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A PROPOSED METHOD FOR THE ESTIMATION OF TOTAL CALCIUM IN SOILS AND THE SIGNIFICANCE OF THIS ELEMENT IN SOIL FERTILITY[1]

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HISTORICAL

The application of lime and limestone to our soils for better crop production has been so beneficial that there is an increasing inquiry as to the probable need of these materials on areas in different sections of our state. The chief reason for their use has been to overcome an apparent acid condition of the soil, which is most easily done by the use of some base, such as caustic, air-slaked or hydrated lime, limestone or dolomite.

Scientific workers are not always in accord as to the factors which cause soil acidity. This is apparent from the fact that the several methods which have been proposed for its estimation do not always agree when applied in practice. It is generally assumed, however, that so-called acidity bears such a close relation to a calcium deficiency that the terms "acidity" and "lime requirement" are practically synonymous.

Very little significance has been attached to the fact that in adding lime or limestone to soils for correcting acidity, an essential element for plant growth is being applied and one which is removed in comparatively large quantities by crops. Hence any material of this nature is commonly classed as a "soil amendment," rather than as a plant-food. The reason of this is, it is assumed that soils generally contain abundant calcium compounds to furnish an ample supply of this element for all crop requirements. As soil-survey work has progressed, however, analyses show that there are certain types of soil in which the small percentage of total calcium found would indicate that there may be a deficiency of this element for permanent fertility. For example, Hopkins (2) from his work in Illinois, concludes that the addition of limestone to those soils which contain less than 3500 pounds of calcium per surface acre of 6⅔ inches in depth, o. 0.175 per cent, has a positive value for the calcium which it supplies as plant-food, in addition to any value in correcting soil acidity or improving

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the physical condition. Van Slyke (7) states that certain of the essential elements are more extensively used by crops and sooner or later require special attention in the way of increasing the available supply in some soils. These elements, given in the order of probable importance, are nitrogen, phosphorus, potassium and calcium. Among others who practically agree with the foregoing are Voorhees (8), Halligan (1) and Thorne (5). As stated before, however, very little importance has been attached to a possible deficiency of calcium in soils, for the reason mentioned above and from the further assumption, possibly, that, calcium compounds being quite soluble, even a limited supply will always furnish the necessary amount for the immediate needs of a crop.

<div align="center">EXPERIMENTAL</div>

In view of the fact that large amounts of limestone are now being used on the soils of our state and the demand is increasing, the writer thought it would be of interest to study the calcium content of our virgin and cultivated soils in order to determine the effect of cultivation on this constituent. This work has been in progress for some time and the data show some interesting results regarding a possible deficiency of this element, in some cases, for normal crop production. This study has embraced both analytical data on soils and pot cultures in the greenhouse, and is still in progress.

The main difficulty that had to be overcome in the work, at first, was in the estimation of calcium. As ordinarily carried on, it readily became apparent that the method at first used, described in this paper as the "regular method," was defective, for the reasons mentioned later, and this necessitated either an improvement of the same or the substitution of another which would be more satisfactory for this determination.

The methods, briefly described, which have been used are as follows:

Regular method. One gram of soil, ground to pass a 100-mesh sieve, was fused with a 5-gm. fusion mixture (10 parts Na_2CO_3 + 13 parts K_2CO_3) for 10 minutes, in a platinum crucible. The melt was dissolved in distilled water, HCl added, the solution evaporated to dryness and the SiO_2 dehydrated by powdering the residue and drying on the water-bath. After dehydration, HCl and H_2O were added and the SiO_2 filtered and washed. The ammonia precipitation was then made in a faintly ammoniacal hot solution and the precipitate filtered and washed once or twice. It was then dissolved in HCl, reprecipitated in the same way and washed with hot water until free of chlorides. The combined filtrates from the ammonia precipitate were then evaporated to low volume, after being made slightly acid with HCl. At this point, bromine water was added, followed by a moderate excess of NH_4OH to precipitate the manganese. After evaporation of excess of NH_4OH, the solution was slightly acidified with $C_2H_4O_2$, the precipitate filtered and washed, only one precipitation of the manganese being made. The filtrate was heated on the bath, a slight excess of NH_4OH added and CaC_2O_4 precipitated with hot, saturated solution of $(NH_4)_2C_2O_4$ and, after standing over night, was filtered, washed a few times, dissolved in HCl and reprecipitated in the same manner.

Modified regular method. The same procedure was used as with the regular method except 2 cc. of 10 per cent $FeCl_3$ solution was added before the ammonia precipitation was made. The first precipitation of the CaC_2O_4 was made in a faintly ammoniacal solution but the reprecipitation was in weak oxalic acid solution.

Sodium peroxide method. One or two grams of 100-mesh soil were fused with 8 gm. Na_2O_2 in an iron crucible. The melt was dissolved in water, acidified with HCl, evaporated and SiO_2 dehydrated. The residue was taken up with HCl and H_2O, filtered and washed until free of chlorides. To the filtrate and washings, in a volume of about 150 cc., was added 0.5 gm. $(NH_4)_2S_2O_8$, the whole heated to boiling and NH_4OH added in excess. After boiling for about 5 minutes, the precipitate was filtered and washed with hot water until practically free of chlorides. The filtrate and washings were then acidified with HCl, evaporated to about 100 cc. 1 or 2 cc. of 6 per cent $FeCl_3$ solution and 0.5 gm. $(NH_4)_2S_2O_8$ added, heated and ammonia precipitation made as before. The filtrate, in a volume of 150 cc., was heated on the bath and hot, saturated $(NH_4)_2C_2O_4$ solution added to precipitate the CaC_2O_4 and allowed to stand over night. The CaC_2O_4 was filtered, washed a few times, dissolved in HCl and reprecipitated in the same manner.

McCrudden method. After making the fusion as in the "regular method," dehydrating and filtering the SiO_2, the calcium in the filtrate was determined by the procedure employed by McCrudden (3, 4) for determining calcium in feces, urine, etc. It was thought that this would not give reliable results with soils and for this reason only a few determinations were made by it.

Preliminary proposed method. The same procedure was followed as in the proposed method to be described later, except the ignition of the CaC_2O_4 and the treatment to remove manganese was omitted. This method was used at the beginning of the work and, unfortunately, no blanks were run, as the importance of these was not then appreciated.

Proposed method. The fusion of the soil and dehydration of SiO_2 was made in the same manner as in the "regular method." The filtrate and washings from the SiO_2 should preferably not exceed 100 cc. Concentrated NH_4OH was carefully added until the solution was just alkaline to litmus, followed by HCl until the litmus paper was just acid. The precipitate at this point in all cases was not entirely dissolved but rather resembled a colloidal ferric hydroxide solution. It was not thought necessary or even desirable to add HCl until the solution was perfectly clear. The solution was then heated to boiling and 1 to 2 gm. of dry powdered $(NH_4)_2C_2O_4$ cautiously added and the heating continued for 2 to 3 minutes. At this point the litmus paper was frequently blue, in which case HCl was added carefully until it was faintly but distinctly acid, the container put on the steam bath for a few hours, and allowed to stand over night at room temperature. The precipitate was filtered until clear, the container carefully washed twice with water, which was poured on the precipitate, and the latter was ignited to convert the calcium oxalate into oxide or carbonate. It was then transferred to the same precipitating container, dissolved in hot dilute HCl, diluted with a small amount of water and heated, after which the manganese was precipitated by the addition of bromine water and a moderate excess of NH_4OH. The heating was continued until only a slight excess of NH_4OH remained, after which the solution was made slightly acid with $C_2H_4O_2$, filtered and the filter washed. This was done to remove manganese which was often present. The filtrate was made faintly alkaline with NH_4OH and then faintly acid with HCl, boiled and about 0.5 gm. $(NH_4)_2C_2O_4$, depending on the amount of calcium present, was added as before, the heating continued for 2 to 3 minutes and the same procedure followed as in the first precipitation.

By this method it is possible for one person to average six determinations a day after the work is begun by working on four sets, each comprising six samples, at the same time. If found more convenient, the time allowed for the precipitation of the CaC_2O_4 may be shortened, as a few minutes' boiling and then allowing to stand for five or six hours on the steam bath will cause complete precipitation. However, it is preferable that the solution be cooled before the filtration is made.

In all methods, it is essential that blanks be run in the same manner as the determinations, the same amounts of chemicals and of water being used, for it has been found here that distilled water prepared from hard water, in the ordinary laboratory still, may contain calcium.

The results obtained both volumetrically and gravimetrically on several soils during the earlier work by these different methods are given in table 1.

TABLE 1

Percentage of total calcium (Ca) in soils found by different methods

SOIL NUMBER	MCCRUD-DEN	SODIUM PEROXIDE		REGULAR		MODIFIED REGULAR	PRELIMI-NARY PROPOSED	PROPOSED
	Vol.	Vol.	Grav.	Vol.	Grav.	Grav.	Vol.	Grav.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
56447	0.212*	0.085	0.200	0.182	0.236	0.129	0.232*	0.186
56447								0.172
56449	0.297*	0.371	0.472	0.373	0.500	0.400	0.444*	0.436
56449								0.429
9768	0.551*	0.615	0.815	0.773	0.879	0.643	0.656*	0.772
9768								0.765
9771	0.127*	0.117	0.307		0.279	0.250	0.211*	0.214
9771								0.207
56485	0.148*	0.386	0.772	0.896	0.707		0.656*	0.572
56487	0.106*	0.197	0.307	0.324	0.329		0.232*	0.279
56457				0.356*	0.457		0.245*	0.236
56459				0.335*	0.400		0.249*	0.293
56517				0.292*	0.386		0.215*	0.272
56519				0.314*	0.357		0.232*	0.250
56521				0.377*	0.443		0.249*	0.229
56523				0.377*	0.393		0.287*	0.257
25662		0.163		0.159	0.372			0.200
25662								0.193
25663		0.108		0.102	0.257			0.143
56493		0.333		0.331	0.550			0.400
56493								0.336
56495		0.201		0.133	0.314			0.207
56513		0.480		0.445	0.672			0.529
56515		0.166		0.138	0.293			0.179

* Determination made on 0.5-gm. aliquots from the same fusion. These particular determinations were made at the beginning when the importance of carrying on blanks in the same manner was partly overlooked. No blanks were run in the McCrudden and preliminary proposed methods but those obtained in the proposed method have been deducted in the preliminary. The McCrudden figures are for the above reason too high but for all other determinations given in this work, the proper blanks have been deducted and unless otherwise stated, were made gravimetrically.

It was apparent at the beginning of the work that the precipitates of CaC_2O_4 obtained by the regular method were not pure, because the CaO, after ignition, was frequently colored and when it was dissolved in HCl and the solution made faintly alkaline with NH_4OH, a precipitate was almost invariably obtained.

The amount of this precipitate, however, varied in different cases, and upon examination it was found to consist mainly of alumina, sometimes with very small amounts of iron, phosphorus and manganese compounds present. This fact has frequently been verified by filtering the precipitates, washing and making a third precipitation of the CaC_2O_4, in which case lower results have always been obtained, especially in those samples which showed perceptible amounts of impurities. Of course, the presence of these impurities causes a plus error but, on the other hand, if calcium is occluded in the ammonia precipitate, which frequently occurs, a compensating minus error is introduced. These errors may or may not balance each other.

The chief source of error in the regular method is due to the hydrolyzation of phosphates in the ammonia precipitate, or to the hydroxide passing through the filter in a colloidal condition on washing with hot water for the removal of the chlorides. Probably this could be largely obviated by the substitution of some salt solution for the water or by not washing entirely free of chlorides, when the ammonia precipitate is not to be weighed. No attempt has been made along these lines in this work, as it was the writer's desire to demonstrate that the regular procedure, as sometimes prescribed for the separation of calcium in soil analysis (9), does not give accurate results. The factor of preventing hydrolyzation in the washing of precipitates containing mixtures of iron and aluminum phosphates and hydrates has been recognized, however, and the use of a salt wash is sometimes prescribed (6).

It is often recommended that a small amount of ferric chloride be added to the solution when the ammonia precipitation is made, in order to obviate the above difficulty, inasmuch as it makes the precipitate more basic in character, especially where aluminium phosphate is involved, as is the case in soils. This has been done in this work and while it undoubtedly is of value in some instances, still it does not always prevent the difficulty, as will be shown later. It might be mentioned here that in no case wherever tested has the CaO obtained by the proposed method ever shown any impurities of this kind that could be recognized by dissolving the same in HCl and neutralizing with NH_4OH, and this is further verified by the fact that the entire amount was recovered in the third precipitation.

The gravimetric results obtained by the regular method, with and without the addition of $FeCl_3$ and subsequently making a third precipitation as above described, are given in table 2. The results obtained from the same samples, by the proposed method, are included for comparison.

It will be seen from an examination of table 2 that the CaO obtained by the regular method, without the addition of $FeCl_3$, generally contained impurities, as was shown by the third precipitation. After the addition of $FeCl_3$, the results, as a rule, were much lower, yet the CaO precipitates were not usually pure, as will be seen in the same table. At the same time, however, the results obtained by a third precipitation agree more closely in several instances with those of the proposed method but are still not altogether satisfactory. The

TABLE 2

Percentage of calcium (Ca); *the regular method, with and without the addition of* FeCl₃, *vs. the proposed method*

SOIL NUMBER	NO FeCl₃ ADDED	FeCl₃ ADDED	THIRD PRECIPITATION	PDOPOSED METHOD
	per cent	*per cent*	*per cent*	*per cent*
14604	1.415	1.393		1.250
14604		1.265		1.293
14604		0.807(a)	0.750(b)	
14604		0.929(c)		
14604		0.886(c)		
14607	0.607(a)		0.429(b)	0.557
14607				0.636
17483	0.536	0.372(a)	0.372(b)	0.407
17483				0.429
25004	0.543	0.314(a)	0.179(b)	0.243
25004				0.200
25549		0.136(a)	0.100(b)	0.107
25549		0.207(c)		0.143
25662	0.372	0.179(a)	0.129(b)	0.200
25662				0.193
36263	0.400	0.079(a)	0.064(b)	0.057
36263				0.071
36264	0.329(a)		0.164(b)	0.057
36264		0.114(a)	0.057(b)	0.036
36264				0.029
36552	0.372	0.236(a)	0.107(b)	0.093
36552				0.057
36552				0.036
56463	0.050(a)		0.021(b)	0.136
56463	0.107(a)		0.043(d)	0.079
56463	0.079(a)		0.036(d)	0.179
56463		0.093(a)	0.100(b)	0.071
56463		0.064(a)	0.043(d)	
56463		0.057(a)	0.014(d)	
36793	0.264(a)		0.079(b)	0.107
36793				0.100
36796		0.457		0.150
36796		0.107(a)	0.121(b)	0.150
56493	0.550	0.372(a)	0.350(b)	0.400
56493				0.336
56494	0.350(a)		0.207(b)	0.214
56494				0.172
56496	0.307(a)		0.200(b)	0.164
56496				0.222
56455	0.400	0.214(a)	0.143(b)	0.200
56455				0.186
56477	0.350	0.071(a)	0.043(b)	0.114
56477				0.100

TABLE 2—*Continued*

SOIL NUMBER	NO FeCl₃ ADDED	FeCl₃ ADDED	THIRD PRECIPITATION	PROPOSED METHOD
	per cent	*per cent*	*per cent*	*per cent*
56506	0.172(a)		0.093(b)	0.043
56506				0.064
56508	0.107(a)		0.100(b)	0.036
56508		0.079(a)	0.043(b)	0.029
56510	0.207(a)		0.136(b)	0.057
56512	0.107(a)		0.064(b)	0.064
56512				0.029
56514	0.414(a)		0.272(b)	0.236
56514				0.293
56458	0.414(a)		0.279(b)	0.214
56458	0.164(a)		0.172(b)	0.222
56458		0.186(a)	0.193(b)	
56460	0.307(a)		0.207(b)	0.143
56460	0.086(a)		0.064(b)	0.150
56460	0.071(a)		0.043(d)	
56460	0.121(a)		0.050(d)	
56460		0.093(a)	0.071(b)	
56460		0.107(a)	0.036(d)	
56460		0.121(a)	0.050(d)	
56473	0.129(a)		0.100(b)	0.243
56473		0.214(a)	0.179(b)	
56497	0.493	0.200(a)	0.200(b)	0.222
56497				0.243

(a) See (b).

(b) The CaO obtained in (a) was dissolved in HCl, made slightly alkaline with NH₄OH and the precipitate, if any was obtained, was filtered, washed and the third precipitation of CaC_2O_4 was made in the filtrate in a faintly ammoniacal solution as before.

(c) Modified regular method used.

(d) The CaO obtained in (a) was treated as in (b) except the third precipitation of CaC_2O_4 was made in faint oxalic acid solution.

low results can be accounted for only as due to the occlusion of calcium in the ammonia precipitates, while the high ones are undoubtedly caused by impurities.

It has been mentioned that calcium has been found to be occluded in the ammonia precipitate and that such was often the case was proved by an examination made of some samples selected at random that had been used in the work. The calcium was determined by the modified regular method and the ammonia precipitate was dissolved in HCl and the calcium in the same was determined by the proposed method, with the results given in table 3.

As the three methods which were mostly used in the work, namely, the regular, modified regular and proposed, gave such discordant figures on many of the soils, it was thought that it might prove of interest to compare them on a synthetic soil mixture which was comparatively high in calcium, phosphorus and aluminium. Accordingly, a sufficient quantity of a mixture of C.P.

chemicals in the required amounts was prepared to represent a soil having the composition given in table 4, in 100 parts, the lacking 71.07 parts being supposed to represent SiO_2, moisture and organic matter. In other words, the 10 parts of SiO_2 used represent only part of that required to make up the supposed soil.

TABLE 3

Percentage of calcium (Ca) occluded in ammonia precipitate in the modified regular method

SOIL NUMBER	MODIFIED REGULAR METHOD	CALCIUM IN AMMONIA PRECIPITATE	TOTAL OBTAINED	PROPOSED METHOD
	per cent	*per cent*	*per cent*	*per cent*
56447	0.129	0.079	0.208	0.186
56447				0.172
56449	0.400	0.057	0.457	0.436
56449				0.429
9768	0.643	0.114	0.757	0.772
9768				0.765
9771	0.250	0.021	0.271	0.214
9771				0.207
14604	0.429	0.529(a)	1.058	1.250
14604		0.100(b)		1.293

(a) and (b), no $FeCl_3$ was added to (a) or the first ammonia precipitation, but to the resulting filtrate was added 2 cc. of 10 per cent $FeCl_3$ solution and the second or (b) was then made.

TABLE 4

Composition of synthetic soil mixture

CONSTITUENT	DERIVED FROM
10.00 per cent SiO_2	0.1000 gm. silica
4.00 per cent Fe_2O_3	0.0524 gm. iron oxide, hydrated
10.00 per cent Al_2O_3*	0.1039 gm. aluminum oxide, hydrated
1.50 per cent P_2O_5	0.0258 gm. aluminum phosphate
0.50 per cent MgO	0.0050 gm. magnesia
1.75 per cent CaO	0.0312 gm. calcium carbonate
0.36 per cent SO_3	0.0101 gm. manganese sulfate
0.32 per cent MnO	0.0101 gm. manganese sulfate
0.50 per cent TiO_2	0.0050 gm. titanium oxide
28.93	0.3334 gm.

* Derived from the oxide and phosphate.

The required amount of the above mixture, or 2.0004 gm. representing 6 gm. of soil, was fused with 5 gm. of the double carbonates as in the regular method. Another equal portion, omitting the $CaCO_3$, was fused in the same manner for the blanks. The amount of SiO_2 added was, of course, much less than that present in a soil but as this made a thorough fusion more possible and at any rate the SiO_2 is eliminated at the beginning, the above quantity was assumed to exceed largely any that might possibly exist in solution after

the dehydration of SiO_2 was made. The quantity of SiO_2 present in the above fusion was therefore about 75 per cent of the amount in 1 gm. of average soil. No sodium or potassium compounds were added, as the fusion mixture supplied these elements. After the fusions were made and the SiO_2 was eliminated the solutions were made to volume and aliquots corresponding to 1 gm. of soil were used for the calcium determinations, by the different methods. In the regular and modified regular methods, $FeCl_3$ was added to one aliquot and omitted in the other. The ammonia precipitates were also examined for calcium as in table 3. The results are given in table 5.

The results obtained on the synthetic mixture by all the methods agree very well with the amount of calcium present, and much better than those obtained when the same methods are applied to soils. This corroborates the writer's previous experience, that a synthetic soil solution does not always behave in the same manner as the soil it is supposed to represent. An examination of table 5 shows that the first two methods gave slightly low results

TABLE 5

Occlusion of calcium in ammonia precipitates in regular and modified regular methods

	CaO BY METHOD	CaO IN AMMONIA PRECIPITATE	TOTAL CaO
	per cent	*per cent*	*per cent*
Regular method, no $FeCl_3$ added........................	1.67	0.05	1.72
Regular method, $FeCl_3$ added..........................	1.70	None	1.70
Modified regular method, no $FeCl_3$ added................	1.68	0.06	1.74
Modified regular method, $FeCl_3$ added..................	1.71	0.01	1.72
Proposed method......................................	1.79		
Proposed method	1.85		
Known content of CaO...............................			1.75

while the proposed method averages a trifle high. In the work on soils, however, the regular method has generally given high results, compared with the proposed method, while the modified method shows both high and low. A tendency also exists here for calcium to be occluded in the ammonia precipitates, which is largely prevented by the addition of $FeCl_3$, but this is not so noticeable as it was in the soils.

During this investigation, only a part of which is included in this paper, a large number of soils have been tested comprising samples taken from nearly every county in the state. In many cases the amount of total calcium found was surprisingly low; in fact the lack of this element in several soils was as noticeable as was their deficiency in phosphorus or nitrogen. Taking into account the amounts of these three elements present, two of which, however, are not reported here, and the quantities of the same removed by crops, all are probably deficient in many of the soils. On the other hand, the potassium content of the most of these soils is relatively high and compares favorably with our best in this respect. Some of the results obtained are given in table 6 and in cases where duplicates were made these are included.

O. M. SHEDD

TABLE 6

Percentage of total calcium (Ca) in soils found by regular, modified regular and proposed methods

SOIL NUMBER	VIRGIN SURFACE 0 TO 6 INCHES			CULTIVATED SURFACE 0 TO 6 INCHES		
	Regular	Modified regular	Proposed	Regular	Modified regular	Proposed
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
56447, 9	0.500	0.400	0.436	0.236	0.129	0.186
56447, 9			0.429			0.172
36552, 91	0.372		0.093			0.071
36552			0.057			
36552			0.036			
56467, 17505	0.615		0.314		0.107	0.007
56467, 17505			0.293			0.036
36538, 9	0.550		0.472	0.336	.	0.279
56469, 71	0.464		0.279	0.279		0.107
56481, 3	0.786		0.407	0.486		0.186
56481, 3			0.386			0.186
56485, 7	0.707		0.572	0.329		0.279
9768, 9	0.879	0.643	0.772			1.458
9768, 9			0.765			1.500
56497, 9	0.615		0.350	0.493		0.222
56497						0.243
56517, 19	0.386		0.272	0.357		0.250
56477, 9	0.350		0.114	0.500		0.214
56477			0.100			
36242, 63	0.400		0.057	0.436		0.172
63			0.071			
56457, 9	0.400		0.293	0.457		0.236
56513, 15	0.672		0.529	0.293	.	0.179
56509, 11	0.243		0.150	0.186		0.129
56493, 5	0.550		0.400	0.314		0.207
56493			0.336			
56505, 7	0.286		0.150	0.229		0.114
56505, 7			0.164			0.057
36792, 6	0.472		0.179	0.457		0.150
36792			0.150			0.150
36919, 20			0.079			0.057
50644, 6			0.293			0.107
50672, 51362			0.207			0.121
25771, 2			0.407			0.186
25787					0.143	0.086
25549					0.207	0.107
25549						0.143
25814					0.179	0.100
36819					0.100	0.107
25828					0.150	0.150
14976					0.064	0.021
14976					0.021	0.050

TABLE 6—*Continued*

SOIL NUMBER	VIRGIN SURFACE 0 TO 6 INCHES			CULTIVATED SURFACE 0 TO 6 INCHES		
	Regular	Modified regular	Proposed	Regular	Modified regular	Proposed
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
17899					0.143	0.114
17341					0.129	0.114
36132					0.136	0.114
17122					0.143	0.157
36507				0.157		0.186
17517						0.029
17517						0.093
17517						0.079
25007						0.021
25007						0.050
25999						0.050
25999						0.071
50904						0.050
50904						0.093
25281						0.029
25281						0.093
25281						0.079
25666						0.043
25666						0.057
50787						0.029
50787						0.079
25262						0.050
25262						0.093
36452						0.093
17901						0.086
25016						0.086
17367						0.100
36493						0.057
36578						0.036

Several virgin soils and the corresponding cultivated samples were used in this investigation in order to determine the loss of calcium due to cultivation. These results, also, are included in table 6.

GENERAL DISCUSSION

The proposed method has given more reliable results in this investigation and has several advantages over the regular procedure. It is more rapid, the calcium precipitates are purer and less difficulty in obtaining duplicates is encountered. It has a further advantage in that the handling of the ammonia precipitate, which is always more or less troublesome and which introduces errors in separations of this character, is avoided.

In an ordinary clay analysis, where small amounts of calcium and of other constituents are to be determined, the ammonia precipitate is difficult to

handle and this is further complicated in a soil because much larger amounts of phosphorus, iron, calcium, manganese and other elements are often present. The chief difficulties in the regular procedure have been the hydrolyzation of ferric or aluminium phosphate in the ammonia precipitate and its passing through the filter in a colloidal form on washing with hot water and the occlusion of calcium, probably in the form of phosphate. It was not found possible in all cases to prevent this by the addition of ferric chloride, as is often recommended, but the substitution of a salt solution, such as ammonium nitrate, instead of hot water, might have been very beneficial. This was not tried, for the reason previously stated, but even if it had been, it could only have prevented hydrolyzation or the formation of a colloidal solution, while the error caused by the occlusion of calcium in the ammonia precipitate would still have to be overcome, especially in those soils which had a large content of calcium phosphate. It should be borne in mind that any method for the estimation of total calcium in soils has its limitations when applied to samples that are comparatively low in this element, for a variation of one-tenth of a milligram in the weight of CaO obtained on a one-gram sample is equivalent to 0.007 per cent of the element. Consequently a variation of a few tenths of a milligram in duplicating, which is probably the best that can be expected on such samples, in work of this character, amounts to considerable on soils which contain only a few hundredths of 1 per cent of this element. Disagreement of duplicates when working on soils of this character cannot be attributed altogether either to the method or individual but, even if such duplicates do not agree closely, they undoubtedly show that the soil is low in this constituent.

From an examination of the foregoing tables, it will be observed that some of our cultivated soils are very low in calcium and frequently this holds true for the corresponding virgin soils. In fact, from other work done on these and similar soils but not reported here, their calcium deficiency assumes equal importance with their low phosphorus and even low nitrogen content. An application of one ton of limestone or of calcium phosphate per acre to such soils frequently supplies more calcium than is already present. In such cases there can hardly be any doubt that the increased plant growth following applications of these materials, or even of some commercial fertilizers, is due, at least in part, to the plant-food calcium which these materials supply, in addition to other good results which they may accomplish.

SUMMARY

1. The procedure which is often used and has been adopted by the Association of Official Agricultural Chemists for the determination of calcium in a soil solution does not give accurate results.

2. The chief difficulties encountered in its use have been due to the passage through the filter of iron and aluminium compounds either from hydrolyzation of ferric and aluminium phosphates in the ammonia precipitate or in a colloidal

condition caused by washing with hot water, and to the occlusion of calcium, probably as the phosphate, in this precipitate.

3. Attempts to prevent these errors by the addition of ferric chloride have not been successful.

4. Other modifications of the above procedure and substitution of· other methods have not been altogether satisfactory for this determination when the process involved separation of the ammonia precipitate previous to the estimation of the calcium.

5. A proposed method which eliminates the chief sources of error of the regular procedure has been found to give more concordant results and is more rapid.

6. When some of the methods used in this work were tried on a synthetic soil solution a better agreement was obtained than when the same were applied to soils. This verifies the author's previous experiences that a synthetic solution does not always act in the same manner as a solution of the soil which it is supposed to represent.

7. The total calcium content of a large number of Kentucky soils, both virgin and cultivated, has been determined, and it appears in nearly every instance that cultivation has caused a considerable loss of this element.

8. From an investigation of which this work is only a part and which has included several hundred samples taken from nearly all the counties in this state, it has been found that the best types of soil contained the highest content of calcium and the poorest had the lowest.

9. Many samples have been found to be so low in calcium that their deficiency in this constituent requires consideration as well as their low phosphorus and nitrogen supply.

10. The application of a ton of limestone or of rock phosphate per acre to such soils frequently adds more calcium than is already present.

11. There is no doubt that, in such cases, these materials, or even moderate applications of some commercial fertilizers, are beneficial because of the plant-food calcium they supply, in addition to other good effects they may accomplish.

REFERENCES

(1) HALLIGAN, J. E.   1912   Soil Fertility and Fertilizers, p. 21.   The Chemical Publishing Co., Easton, Pa.

(2) HOPKINS, C. G., ET AL.   1911   Moultrie County soils.   Ill. Agr. Exp. Sta. Soil Rpt. 2, p. 38.   Also see succeeding Soil Reports.

(3) McCRUDDEN, F. H.   1910   The quantitative separation of calcium and magnesium in the presence of phosphates and iron.   In Jour. Biol. Chem., v. 7, p. 83–100.

(4) McCRUDDEN, F. H.   1911   The determination of calcium in the presence of magnesium and phosphates; the determination of calcium in urine.   In Jour. Biol. Chem., v. 10, p. 187–199.

(5) THORNE, C. E.   1914   Farm Manures, p. 57.   Orange Judd Co., New York.

(6) TREADWELL, F. P., AND HALL, W. T. 1905 Analytical Chemistry, 1st ed., v. 2, p. 392. John Wiley and Sons, New York.
(7) VAN SLYKE, L. L., 1912 Fertilizers and Crops, p. 21. Orange Judd Co., New York.
(8) VOORHEES, E. B., 1903 Fertilizers, 5th ed., p. 3. The MacMillan Co., New York.
(9) WILEY, H. W., editor 1908 Official and provisional methods of analysis of the Association of Official Agricultural Chemists, U. S. Dept. Agr. Bur. Chem. Bul. 107, revised, p. 15.

THE USE OF CARBON BISULFIDE AGAINST THE WHITE GRUB[1]

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INTRODUCTION

The control of soil-infesting insects has always been an exceedingly vexatious problem for the economic entomologist. Their subterranean feeding habit renders them immune to the ordinary methods of control by stomach poisons or contact insecticides, while in many cases the 2- or 3-year life cycle adds another complication to an already complex problem. The usual recommendations for the control of such pests are crop rotation and fall plowing.[3] These methods, while quite applicable to crops which mature in one growing season, are impracticable when infestation occurs on plants or crops of a more permanent nature. In New Jersey the greatest recent injury has occurred in lawns, golf-greens and strawberry beds. In lawns and golf-greens the nature of the injury is such that the roots of the grass are eaten off just below the surface of the ground. In strawberry beds the injury is done when the plants are set out in land which was previously in sod; in this case the roots of the plants are cut off below the crown, resulting in extreme cases in the loss of the entire bed. For this injury the common white grubs, larvae of *Lachnosterna* beetles, are responsible. In order to effect a feasible control of these insects when present in such locations, some method must be devised which will exterminate the insect, and at the same time not injure the plant it is infesting. The method chosen for this purpose was soil fumigation with carbon bisulfide. The problem to be solved was to determine whether a dosage could be found which would be fatal to the grub, and at the same time not injure the plant. The problem was divided into three parts, the determination of the maximum dosage non-injurious to the plant, the determination of the minimum dosage lethal to the grub, and the determination of the influence of temperature and moisture conditions upon the effectiveness of the fumigation.

[3] Since this paper was written sodium cyanide has been successfully used against white grubs by F. A. Kaufman of the Roessler and Hasslacher Chemical Company, by W. H. Goodwin and finally by J. J. Davis of the Riverton Entomological Laboratory.

The material for the studies on the control of the white grub was obtained during the fall of 1915. The grubs were dug from an uncultivated field in soil where they were found at the roots of grass and weeds. The material was preserved alive according to the method given by J. J. Davis (1). Briefly outlined, this method consists of placing a single grub in a small tin ointment box, with moist earth, and carefully sterilized wheat which has been deprived of germinating power by heat. The necessity for killing the wheat and sterilizing it was shown by previous experience, when the wheat kernels germinated inside the boxes, and fungus spores attacked the wheat in other boxes, rendering it unfit for larval food. By this method of preservation, larvae were kept alive and vigorous from October, 1915, to May, 1916.

DETERMINATION OF THE MAXIMUM DOSAGE NON-INJURIOUS TO PLANTS

The first phase of the problem, the determination of the maximum dosage non-injurious to plants, was attacked by trying the effect of carbon bisulfide on plants grown in the greenhouse. The flats in which the plants were grown

TABLE 1

Temperature of soil and of air—first maximum dosage experiment

DATE	AIR		BLUEGRASS			BLUEGRASS AND CLOVER		
	Maximum	Minimum	Soil dry	Soil medium	Soil wet	Soil dry	Soil medium.	Soil wet
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
2- 5-16	92	68	64	64	63	71	70	64
2- 7-16	73	65½	73	75	72	72	71	70
2- 8-16	65½	47	63	63	61	65	64	62
2- 9-16	83½	58	67	66	63½	70	72	62
2-10-16	83	60	69	70	73	70	73	71
2-11-16	80	58	57	61	61½	60	60	60
2-12-16	80	57	58	60	61	60	60	61
2-14-16	60	45	50	49	50	49	48	49

were 31 by 15 inches and 6 inches deep. Inasmuch as lawns are chiefly made up of a mixture of Kentucky bluegrass and white clover, six flats were planted: two flats each of bluegrass, of white clover and of a mixture of the two. These flats were prepared for the experiments by enclosing a section having an area of 1 square foot, and a depth of 5 inches (the depth of the greenhouse bench) in a square casing of galvanized iron, which was driven down to the bottom of the bench. Carbon bisulfide (Lehn and Fink, technical) was introduced into each of three flats, one flat each of bluegrass, clover, and a mixture of the two, at the rate of 2 ounces to each section. The dose was introduced by means of a pipette into a hole 3 inches deep placed in the center of the section. Soil and air temperatures are recorded in table 1. Determinations of the actual soil moisture were not made, but it is thought that they were about the same as shown in later experiments on page 23.

A month after the introduction of the carbon bisulfide no apparent injury had been done to any of the plots with the 2-ounce charge of carbon bisulfide, except for the very slight contact kill incidental to the introduction of the material. This experiment demonstrates that under the above conditions, 2 ounces of carbon bisulfide may be used on bluegrass, white clover, or a mixture of the two, without injury to the plants.

As a continuation of the above experiments, six flats of the same dimensions as those used in the preceding experiment, containing timothy, red clover,

TABLE 2

Temperature of soil and of air—second maximum dosage experiment

DATE	AIR		TIMOTHY		CLOVER		TIMOTHY AND CLOVER	
	Maximum	Minimum						
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
12- 4-15	66	54	53	53	47	47	54	54
			50	50	51	51	55	54
12- 6-15	62½	50	50	50	46	46	50	50
			49	49	48	48	51	51
12- 7-15	69	50	54	54	49	50	54	54
			51	51	53	53	56	65
12- 8-15	72	46	55	55	54	54	53	54
			54	54	55	55	55	55
12- 9-15	72	48	55	55	52	52	56	55
			54	53	53	53	56	56
12-10-15	73	47	61	61	59	59	61	61
			60	60	60	60	61	60
12-11-15	71½	48	60	60	58	58	60	59
			61	61	59	59	59	59
12-13-15	73	52	61	62	60	61	62	62
			62	62	61	61	61	62

and a mixture of the two, were divided into two parts by a board partition. Thus, four plots of each mixture were formed.

The plots of timothy, red clover and the mixture of the two designated as No. 1 received 2 ounces of carbon bisulfide; those designated as No. 2, No. 3 and No. 4 received 1½ ounces, 1 ounce, and ½ ounce, respectively. Maximum and minimum temperatures were taken daily except Sundays. Table 2 below gives the soil and air temperatures.

Eleven days after the introduction of the carbon bisulfide an examination of the plots for injury was made. The results are given in table 3.

As conclusions to be drawn from the experiment, at a temperature of 60°F. or below, 1 ounce of carbon bisulfide has no deleterious effect on such plants as timothy and red clover. Amounts in excess of 1 ounce are slightly injurious to clover, but injury to timothy was negligible. In all these experiments on plants, the plants were at the most only 6 weeks or 2 months old, and were vigorously growing. In other words, their rate of metabolism was very high, and they were in a favorable condition for injury to occur from the use of so toxic a material as carbon bisulfide.

The next experiment to determine the lethal dosage for plants duplicated the method used in the first experiment with Kentucky bluegrass and white clover. Plants of the same kind were used, being placed under as nearly

TABLE 3 *

Effect of carbon disulfide applications—maximum dosage experiments

PLANT	NUMBER OF PLOT	DOSAGE	REMARKS
		ounces	
Timothy........	1	2	Slight injury at point of introduction
	2	1½	Injury less than preceding
	3	1	No apparent injury
	4	½	No apparent injury
Red clover.....	1	2	Injury greatest at point of application. Many plants killed
	2	1½	Injury less
	3	1	No perceptible injury
	4	½	No injury
Timothy and red clover....	1	2	Few timothy plants killed. All clover dead near hole
	2	1½	Clover suffered more than timothy, which was little injured
	3	1	Scattering injury to clover, none to timothy
	4	½	Clover slightly injured. No injury to timothy

similar conditions of soil moisture as possible, but the temperature was not controlled, as this was impracticable under greenhouse conditions. In this experiment the effect of higher dosages with carbon bisulfide was tried. A uniform dose of 5 ounces to the square foot was applied in the same manner as in the preceding experiment. Soil and air temperatures were taken daily and are given in table 4.

The moisture of the soils at the beginning of the experiment was: dry 5.29 per cent, medium 10.73 per cent, wet 21.37 per cent on the bluegrass plots; on the mixed plots it was: dry 6.07 per cent, medium 12.97 per cent, wet 19.56 per cent.

Three weeks after the beginning of the experiment, no injury was apparent to any of the plots except one of bluegrass. This plot received exactly the same treatment as all the other plots, and no reason can be detected for the

effect of the carbon bisulfide in this case, if indeed the carbon bisulfide was responsible for the injury. The bluegrass in the plot containing the mixture of bluegrass and white clover was ininjured.

According to these results apparently 5 ounces of carbon bisulfide can be used under the above conditions without injury to a mixture of bluegrass and white clover.

Experiments performed outdoors on a lawn seem to show that a dosage of 5 ounces is injurious to grass. A plot of grass near the greenhouse was selected, and a series of holes made with a dibble. Six rows of four holes each were made, with the holes one foot apart in each row and the rows one foot apart. In the first and fourth rows the holes were 3 inches deep; in the second and

TABLE 4

Temperature of the soil and of the air—first lethal dosage experiment

DATE	AIR		BLUEGRASS			BLUEGRASS AND CLOVER		
	Maximum	Minimum	Soil dry	Soil medium	Soil wet	Soil dry	Soil medium	Soil wet
	°F.	°F.	°F.	°F.	F.	°F.	°F.	°F.
3-11-16	92	54	63	64	62	63	62	63
3-12-16	86	60	70	68	71	72	70	70
3-13-16	75	53	72	73	69	73	70	70
3-15-16	78	52	73	73	72	73	69	68
3-16-16	70	41	70	68	66	69	70	67
3-17-16	83	47	74	72	70	72	70	70
3-18-16	86	42	69	69	64	70	68	66
3-20-16	86	51	69	70	69	71	68	67
3-22-16	93	51	74	72	72	75	71	70

fifth rows 6 inches deep, and in the third and sixth rows 12 inches deep. A line passed through the center of each row divided the plot into two equal parts, one of which was watered freely, while the other was not watered.

The first three rows, consisting of twelve holes, received a charge of 1 ounce per hole, while the last three rows received a charge of 5 ounces per hole. About 2 weeks after the charge had been placed, the plot was examined, and it was found that while there was no injury to the grass which surrounded the holes in which a 1-ounce charge had been placed, all the grass for a distance of approximately 4 inches from the hole had been killed by the application of 5 ounces. Neither the depth of the hole nor the moisture condition seemed to influence the result, as the killing was quite uniform with a 5-ounce charge. With the facts ascertained previously in hand, it was not deemed necessary to use a greater charge than 5 ounces, as the lethal dose appeared to be somewhere in the neighborhood of 5 ounces or less. The soil temperature at the beginning of the experiment was 63°F. and did not vary greatly during the week next succeeding the application.

MINIMUM DOSAGE LETHAL TO THE GRUB

The second phase of the problem, the determination of the minimum dosage lethal to the grub, was attacked by placing the grubs in small wire cylinders, which were buried at varying depths in the soil of the greenhouse flats; the flats in turn were treated with varying doses of carbon bisulfide.

The type of soil used in these experiments was the red shale decomposition product known as Penn loam, a heavy, red, clayey soil, baking readily on drying, and cracking after being puddled. Four flats were used, twelve grubs ·

TABLE 5

Temperature of soil and of air—second lethal dosage experiment

DATE	AIR		SOIL		
	Maximum	Minimum	Dry	Medium	Wet
	°F.	°F.	°F.	°F.	°F.
2-9-16	83.5	58	68½	70	66½
			72	67	66
			72	70	62
2-10-16	83	60	68½	68½	65
			72	69	64½
			71½	69½	64
2-11-16	80	58	67½	65	57
			69	62½	56
			69	67½	58
2-12-16	70	58	62	59	60
			61½	56½	57
			62	60	58
2-13-16	80	57	Sunday		
2-14-16	61	42	51	50	50
			51	49	48
			51	47	46

being placed in each flat. The first flat received 1 ounce, the second 0.5 ounce, the third 0.1 ounce, and the fourth, a check, received no treatment. The grubs were placed in cylindrical galvanized iron wire cages 4 inches long and 1½ inches in diameter. Two grubs, separated from each other by a cotton plug, were placed in each cage, together with wheat grains for food.

The cages were placed in the soil at two distances from the point of injection of the carbon bisulfide, and at two depths from the surface. Two cages were placed at a distance of 12 inches from the point of injection, on opposite sides of the point, one at a depth of 1 inch and the other at 5 inches below the soil surface. Four others were placed 6 inches from the point of injection,

90° apart, so arranged that of each pair of cages 180° apart, one cage was 1 inch below the surface, and the other 5 inches below the surface of the soil.

The carbon bisulfide was injected into the soil by means of a pipette, at a distance of 3 inches below the surface.

Soil and air temperatures were recorded daily during the course of the experiment, and are given in table 5.

The conclusions to be drawn from this experiment are that the minimum dose for the white grub lies in the neighborhood of 1 ounce per square foot, at an average temperature of 60° to 65°F., and that deeper-lying forms, provided the depth does not exceed 6 inches, are much more strongly affected than the shallow-lying individuals.

TABLE 6

Effect of carbon bisulfide on white grubs—lethal dosage experiments

DOSE	DISTANCE	DEPTH	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*				*per cent*
0.1	12	5	2	0	2	0
0.1	12	1	2	0	2	0
0.1	6	5	4	0	4	0
0.1	6	1	4	0	4	0
0.5	12	5	2	2	0	100
0.5	12	1	2	0	2	0
0.5	6	5	4	4	0	100
0.5	6	1	4	2	2	50
1.0	12	5	2	2	0	100
1.0	·12	1	2	0	2	0
1.0	6	5	4	4	0	100
1.0	6	1	4	2	2	50

THE INFLUENCE OF TEMPERATURE UPON THE EFFECTIVENESS OF THE FUMIGATION

A series of experiments was next performed to determine the influence of temperature upon the effectiveness of fumigation. The first step was to determine the amount of carbon bisulfide which, when vaporized in air at a known temperature, would constitute a sublethal dose; in other words, a charge which would almost but not quite kill the grubs exposed to it. This determination was necessary in order to give a starting-point from which to determine whether an increase in temperature with the same dose would result in any increase in the effectiveness of the fumigant.

To determine this sublethal dose, grubs were placed in 2-liter Mason jars, which could be hermetically sealed by means of a rubber ring and a glass cap. Varying amounts of carbon bisulfide were placed in the jars, and at the end of 24 hours the grubs were removed and examined for signs of life. It was found that the fumigant had a stupefying effect in cases where it did

not kill outright, for many grubs which, when first removed from the jars, showed no signs of life were very active 24 hours after removal from the jars containing the fumigant. In this manner it was found that at a tempera- ture of 65°F., 5 drops, or approximately 0.2 cc., of carbon bisulfide in 2000 cc. of air will not kill, while 10 drops in 2000 cc. was fatal to all grubs sub- jected to it. The experiment was checked through twice in order to make sure of the dosage.

The experiment was then tried on grubs in the soil. The volume of air remaining in a container, when filled as completely as might be with soil, was determined approximately by displacing the air with water. In this way it was determined that the container held about 425 cc. of air in addition to the soil it contained when full. On this basis, fumigation in air-tight containers in chambers in which the temperature could be maintained constant was tried. The necessary temperature was maintained by electric-light bulbs of different filaments and candle-power, contained in the chambers. The tem- perature was first regulated in the chambers before placing the grubs in the containers, and then the grubs were placed in the soil and the containers sealed. The larvae were allowed to become acclimatized for 24 hours before introducing the carbon bisulfide. One drop of the fumigant to each container was used, corresponding to one drop to each 425 cc. of air. This in turn corresponds closely to the dosage determined before as not fatal to the grub at a temperature of 65°F. A series of temperatures was run, beginning at 65°F. and including 75°F., 85°F., 95°F. and 105°F. The temperature of 105°F. could not be used in a fumigation experiment, as of itself it was sufficient to kill the grubs in 24 hours without the addition of carbon bisulfide.

The results of the experiment were as follows:

At 65° F. one drop of CS_2 in 425 cc. of air will *not* kill.
At 75° F. one drop of CS_2 in 425 cc. of air will *not* kill.
At 85° F. one drop of CS_2 in 425 cc. of air *will* kill.
At 95° F. one drop of CS_2 in 425 cc. of air *will* kill.
At 105° F. the grubs were killed by the heat effect alone.

Thus it is shown that the effectiveness of the fumigation is dependent upon the temperature, being more effective at the higher temperatures. This is in accord with the literature in regard to fumigation against other insect pests.

THE INFLUENCE OF MOISTURE UPON THE EFFECTIVENESS OF FUMIGATION

In this series of experiments the effect of soil moisture was investigated, to discover any relationship between soil moisture and effectiveness of fumi- gation. The soil type used was the same as that in the experiments upon the minimum dosage for the grubs (page 20). Three soil-moisture conditions were used, being determined according to the Official Methods for Soil Anal- ysis, as follows: wet, moisture 24.72 per cent, medium moisture 12.3 per cent,

and dry moisture 4.67 per cent.   Three charges of carbon bisulfide were used, 0.1 ounce, 0.5 ounce, and 1 ounce.   Twelve flats were used, each 15 by 30 inches and 6 inches deep, all filled with the same type of soil.   These were divided into four series of three flats each, on a basis of the dose received. The first series of three flats were treated with the 0.1-ounce charge, the second with the 0.5-ounce charge, and the third with the 1-ounce charge, while the fourth, a check, received no treatment.   In each of these series of three flats, one flat contained dry soil, the second medium-moist soil, and the third, wet soil.   The grubs were placed in cylinders of the same kind as described under the heading "Minimum dosage lethal to the white grub."

TABLE 7

Temperature of the soil and of the air—moisture experiments, first trial

DATE	AIR		SOIL		
	Maximum	Minimum	Dry	Medium	Wet
	°F.	°F.	°F.	°F.	°F.
2- 9-16	83½	58	68½	70	66½
			72	67	66
			72	70	62
2-10-16	83	60	68½	68½	65
			72	69	64½
			71½	69½	64
2-11-16	80	58	67½	65	57
			69	62½	56
			69	67½	58
2-12-16	70	58	62	59	60
			61½	56½	57
			62	60	58
2-13-16	80	57	Sunday		
2-14-16	61	42	51	50	50
			51	49	48
			51	47	46

The arrangement of the cages was the same as described under that heading.   The carbon bisulfide was injected into the soil by means of a pipette, at a distance of 3 inches below the surface.

Soil and air temperatures were recorded daily during the course of the experiment, and are given in table 7.

The record for the dry plot is presented in table 8.

Table 9 shows the record on the medium-moisture plot.

The record on the wet plot is given in table 10.

The experiments were repeated in order to determine whether any effect could be obtained by trying to keep the carbon bisulfide within the soil by means of a blanket of moisture, which would tend to prevent the rapid diffusion upward through the surface layers of the soil. All conditions for this experiment duplicated as nearly as possible those of the preceding, the soil

TABLE 8

Effect of carbon bisulfide on white grubs in the dry plot—first trial

DOSE	DISTANCE	DEPTH	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*				*per cent*
0.1	12	5	2	0	2	0
0.1	12	1	2	0	2	0
0.1	6	5	4	0	4	0
0.1	6	1	4	0	4	0
0.5	12	5	2	0	2	0
0.5	12	1	2	0	2	0
0.5	6	5	4	0	4	0
0.5	6	1	4	0	4	0
1.0	12	5	2	0	2	0
1.0	12	1	2	0	2	0
1.0	6	5	4	2	2	50
1.0	6	1	4	0	4	0

The average soil temperature in the dry plot, with a dose of 0.1 ounce, was 63.5°F.
The average soil temperature in the dry plot, with a dose of 0.5 ounce, was 65.1°F.
The average soil temperature in the dry plot, with a dose of 1 ounce, was 65.1°F.

TABLE 9

Effect of carbon bisulfide on white grubs in the medium-moisture plot—first trial

DOSE	DISTANCE	DEPTH	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*				*per cent*
0.1	12	5	2	0	2	0
0.1	12	1	2	0	2	0
0.1	6	5	4	0	4	0
0.1	6	1	4	0	4	0
0.5	12	5	2	0	2	0
0.5	12	1	2	0	2	0
0.5	6	5	4	0	4	0
0.5	6	1	4	1	3	25
1.0	12	5	2	0	2	0
1.0	12	1	2	0	2	0
1.0	6	5	4	0	4	0
1.0	6	1	4	0	4	0

The average soil temperature in the medium-moisture plot was 62.5°F., with a charge of 0.1 ounce.
In the plot with 0.5 ounce the average soil temperature was 59°F.
In the plot with the 1-ounce charge the average temperature was 63°F.

moisture being the same within a fraction of 1 per cent, the only difference being in the amount of carbon bisulfide which was injected into the flats. These new dosages were 0.5 ounce, 1 ounce, and 1.5 ounce. Immediately after introducing the carbon bisulfide, the soil was blanketed with moisture

TABLE 10

Effect of carbon bisulfide on white grubs in the wet plot—first trial

DOSE	DISTANCE	DEPTH	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*				*per cent*
0.1	12	5	2	0	2	0
0.1	12	1	2	0	2	0
0.1	6	5	4	0	4	0
0.1	6	1	4	0	4	0
0.5	12	5	2	2	0	100
0.5	12	1	2	0	2	0
0.5	6	5	4	4	0	100
0.5	6	1	4	2	2	50
1.0	12	5	2	2	0	100
1.0	12	1.	2	0	2	0
1.0	6	5	4	4	0	100
1.0	6	1	4	2	2	50

The average soil temperature in the plot receiving 0.1 ounce was 63.5°F.
The average soil temperature in the plot receiving 0.5 ounce was 62°F.
The average soil temperature in the plot receiving 1 ounce was 64°F.

TABLE 11

Temperature of the soil and of the air—moisture experiments, second trial

DATE	AIR		SOIL		
	Maximum	Minimum	Dry	Medium	Wet
	°F.	°F.	°F.	°F.	°F.
3-11-16	92	54	79	80	70
			71	73	70
			72	78	71
3-13-16	86	60	70	77	69
			68	71	69
			67	69	70
3-14-16	75	53	66	62	68
			65	68	64
			63	62	62

sprinkled on gently over the surface of all the plots. Air and soil temperatures were recorded daily, and are presented in table 11.

The records on the plots are given in tables 12, 13 and 14.

A dry check was run, receiving no fumigation. All grubs were alive when examined at the end of the experiment. ·

TABLE 12

Effect of carbon bisulfide on white grubs in the dry plot—second trial

DOSE	DISTANCE	DEPTH	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*				*per cent*
0.5	12	5	2	0	2	0
0.5	12	1	2	0	2	0
0.5	6	5	4	3	1	75
0.5	6	1	4	0	4	0
1.0	12	5	2	0	2	0
1.0	12	1	2	0	2	0
1.0	6	5	4	3	1	75
1.0	6	1	4	0	4	0
1.5	12	5	2	0	2	0
1.5	12	1	2	0	2	0
1.5	6	5	4	3	1	75
1.5	6	1	4	0	4	0

TABLE 13

Effect of carbon bisulfide on white grubs in the medium plot—second trial

DOSE	DISTANCE	DEPTH	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*				*per cent*
0.5	12	5	2	0	2	0
0.5	12	1	2	0	2	0
0.5	6	5	4	0	4	0
0.5	6	1	4	0	4	0
1.0	12	5	2	1	1	50
1.0	12	1	2	0	2	0
1.0	6	5	4	4	0	100
1.0	6	1	4	0	4	0
1.5	12	5	2	1	1	50
1.5	12	1	2	1	1	50
1.5	6	5	4	4	0	100
1.5	6	1	4	3	1	75

TABLE 14

Effect of carbon bisulfide on white grubs in the wet plot—second trial

DOSE	DISTANCE	DEPTH	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*				*per cent*
0.5	12	5	2	0	2	0
0.5	12	1	2	0	2	0
0.5	6	5	4	1	3	25
0.5	6	1	4	0	4	0
1.0	12	5	2	1	1	50
1.0	12	1	2	0	2	0
1.0	6	5	4	3	1	75
1.0	6	1	4	1	3	25
1.5	12	5	2	0	2	0
1.5	12	1	2	1	1	50
1.5	6	5	4	4	0	100
1.5	6	1	4	3	1	75

In order to check against the possibility of diffusion of the carbon bisulfide downward through the bottom of the wooden flats used, a determination of the killing effect of carbon bisulfide was made in two galvanized iron pans, each having the same dimensions as the wooden flats used in the experiments. In this experiment two degrees of soil moisture were used, very dry (3.97 per cent moisture) and wet (24.03 per cent moisture). The soil was the same as that used in the other experiments in the wooden flats. The dosage was 1 ounce. Grubs in galvanized wire cages were placed as in the previous experiments. Soil and air temperatures are given in table 15 and the results in table 16.

TABLE 15

Temperature of the soil and of the air—experiment in iron pans

DATE	SOIL MOISTURE	SOIL TEMPERATURE	AIR TEMPERATURE
		°F.	°F.
4-15-16	Wet	80	
	Dry	84	88
4-17-16	Wet	76	
	Dry	80	84

TABLE 16

Effect of the fumigation on white grubs

DOSE	DISTANCE	DEPTH	SOIL	NUMBER PLACED	NUMBER DEAD	NUMBER ALIVE	MORTALITY
ounces	*inches*	*inches*					*per cent*
1	12	5	Wet	2	1	1	50
1	12	1	Wet	2	0	2	0
1	6	5	Wet	4	4	0	100
1	6	1	Wet	5	4	0	100
1	12	5	Dry	2	2	2	0
1	12	1	Dry	2	1	1	50
1	6	5	Dry	4	4	0	100
1	6	1	Dry	4	3	1	75

CONCLUSIONS

Thus it appears that the maximum dosage for ordinary lawn and golf-green grasses lies somewhere between 1 and 5 ounces per square foot and considerably above the former, while the minimum dosage for the white grub is about 1 ounce. Temperature is shown to exert a decided influence on the minimum dosage for the white grub (1 ounce at 65°F. and less than 1 ounce being necessary at 85°F. or above) and presumably also upon the maximum dosage for the plants. Effective work against the grub appears to require injections not much over 6 inches apart. The soil moisture must be medium (10 per cent) to wet (20 per cent) for good results in grub destruction. Wetting the

surface of the soil in cases when the moisture is dry (5 per cent) to medium (10 per cent) seems to increase the effectiveness of the treatment. The charge should be placed several inches below the point where the grubs are working.

In general it may be said that this study seems to show that the control of the white grub when it occurs in situations in which it cannot be reached practically by cultural methods may be effected by fumigation of the soil by means of carbon bisulfide. The combination of its effectiveness against the white grub, its non-poisonous effect on plants when used in small quantities, and the stimulating effect which small dosages have upon lawn vegetation, make it a promising means of control for the white grub. Its relatively high cost will prove a limiting factor.

REFERENCE

(1) DAVIS, J. J. 1915 Cages and methods of studying underground insects. *In* Jour. Econ. Ent., v. 8, no. 1, p. 135-139.

THE OXIDIZING POWER OF SOIL FROM LIMED AND UNLIMED PLOTS AND ITS RELATION TO OTHER FACTORS[1]

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INTRODUCTION

In the spring of 1908 a group of forty $\frac{1}{20}$-acre plots were laid out on the New Jersey Agricultural Experiment Station farm for the purpose of studying the influence of lime and of fertilizers upon the soil and upon the crops. As shown by the report of Lipman and Blair (3) one-half of these plots have received various fertilizer and manurial treatments, together with lime applications, while the other half received the same treatment but without lime. Different amounts of lime have been added to another group of 28 plots, the checks being unlimed (5). For over ten years a careful record of yields of dry matter, nitrogen recovery and chemical changes in the soil have been kept for these plots. Biochemically, a study of the oxidizing power of these soils is of especial interest because the chemical analyses show that the limed plots have lost considerably more carbon than those which were not limed.

HISTORY OF THE PLOTS[2]

Four of the plots which will be designated as 11A, 11B, 21 and 24 were selected for the biological work reported herein. The drainage of the field in which these plots are located appears to be quite uniform. The soil has been classified as a Sassafras loam. It contains a small amount of coarse gravel and has a maximum water capacity of 49.8 per cent. Those who selected the land for these plots (3) state that it had been used for general farming for many years and that it has not been limed for a long period, probably from 20 to 25 years.

Plot 11A receives an annual application of minerals, consisting of acid phosphate at the rate of 640 pounds, potassium chloride at the rate of 320 pounds and ammonium sulfate equivalent to 320 pounds of nitrate of soda per acre. Plot 11B receives a similar application, together with 4000 pounds per acre of ground limestone once in 5 years. Plots 21 and 24 receive annual appli-

[1] Technical paper No. 4 of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] Prof. A. W. Blair has kindly submitted data for the crop and soil history of these plots and has also read the manuscript.

cations of acid phosphate, potassium chloride and sodium nitrate at the rates
of 400, 200 and 200 pounds, respectively. In addition, plot 24 received ground
limestone applications as in plot 11B. It should be stated that the first lime
application (1908) was at the rate of only 2000 pounds per acre.

Plots 11A and 11B have been subjected to a 5-year rotation consisting of
corn 1 year, oats 1 year, wheat 1 year and timothy 2 years. The rotation
on plots 21 and 24 has been corn with vetch as a green-manure crop the
first year, oats followed by cowpeas as a green-manure crop the second year,
wheat the third year, and timothy and clover the fourth and fifth years. Thus

TABLE 1

The total dry matter during a 10-year period, in crops from limed and unlimed fertility plots

YEAR	CROP	PLOT 11A UNLIMED		PLOT 11B LIMED		CROP	PLOT 21 UNLIMED		PLOT 24 LIMED	
		Grain	Hay, straw or stover	Grain	Hay, straw or stover		Grain	Hay, straw or stover	Grain	Hay, straw or stover
		lbs.	lbs.	lbs.	lbs.		lbs.	lbs.	lbs.	lbs.
1908	Corn	134.0	169.7	147.6	177.9	Corn	78.2	145.5	100.9	159.3
1909	Oats	24.5	100.4	14.5	104.5	Oats	16.6	59.7	22.2	73.6
1910	Oats	85.0	177.5	87.5	170.0	Wheat	50.0	92.5	72.3	132.5
1911	Wheat	75.0	97.5	85.0	110.0	Oats	42.5	62.5	50.0	75.0
1912	Timothy		146.3		120.0	Timothy aud clover		96.3		108.8
1913	Corn	130.0	182.5	255.0	310.0	Corn	85.0	265.0	167.5	315.0
1914	Oats	80.0	140.0	50.0	126.0	Oats ·	66.0	86.0	60.0	102.0
1915	Wheat	128.0	180.0	160.0	248.0	Wheat	104.0	124.0	124.0	148.0
1916	Timothy		240.0		408.0	Timothy and clover	·	380.0		600.0
1917	Timothy		192.0		354.0	Timothy and clover		262.0		350.0
Total dry matter		656.5	1625.9	799.6	2128.4		442.3	1573.5	596.9	2064.2
Per cent increase over unlimed plots		20.2	30.9				34.9	31.2		

it is seen that the rotation on plots 21 and 24 has included legumes, while
that on plots 11A and 11B has not.

The dry weights of the crops of these four plots for a period of 10 years
or two rotations are given in table 1. With few exceptions, the annual yield
of grain and of straw or of stover is higher on the limed than on the unlimed
plots. When the total yields for 10 years are considered, the yields of grain
on the limed plots 11B and 24 are greater by 20.2 and 34.9 per cent, respec-
tively, than those of the unlimed plots 11A and 21. In the same way the
yields of hay, straw and stover are 30.9 and 31.2 per cent greater on the limed
than on the unlimed plots.

The average yield of dry matter of the 40 plots, which include plots 11A and 11B is about the same on the limed as on the unlimed plots. Lipman and Blair (4) state that "these experiments would seem to show beyond a doubt, that for the light coastal plain soils, lime has very little place in rotations which omit legumes." Plots 11A and 11B are the only ones of the above series to which ammonium sulfate was added. This explains why lime increased the crop yield, as the soil on plot 11A is now in a very poor condition.

Although lime has a marked effect upon increasing the dry weight of the crops, it does not influence the nitrogen content appreciably. The averages

TABLE 2

The percentage of total nitrogen, during a 10-year period, in dry matter of crops taken from limed and unlimed fertility plots

YEAR	CROP	PLOT 11A UNLIMED		PLOT 11B LIMED		CROP	PLOT 21 UNLIMED		PLOT 24 LIMED	
		Grain	Hay, straw or stover	Grain	Hay, straw or stover		Grain	Hay, straw or stover	Grain	Hay, straw or stover
		per cent	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
1908	Corn	1.48	0.95	1.42	0.97	Corn	1.31	0.50	1.29	0.59
1909	Oats	2.37	1.09	2.26	1.20	Oats	2.20	0.86	2.26	0.84
1910	Oats	2.04	0.58	1.95	0.65	Wheat	1.91	0.45	1.76	0.42
1911	Wheat	2.55	0.63	2.30	0.50	Oats	2.12	0.97	2.18	0.81
1912	Timothy		0.93		0.94	Timothy and clover		0.85		0.89
1913	Corn	1.52	0.82	1.48	0.88	Corn	1.21	0.57	1.44	0.82
1914	Oats ·	2.34	0.97	2.39	1.02	Oats	2.02	0.73	2.19	0.75
1915	Wheat	2.34	0.68	2.08	0.45	Wheat	1.97	0.30	1.99	0.28
1916	Timothy		1.09		0.83	Timothy and clover		0.87		1.20
1917	Timothy		1.03		0.76	Timothy and clover		0.76		0.85
Average per cent		2.09	0.88	1.98	0.82		1.82	0.67	1.87	0.75

for the 10 years show a slightly higher percentage for grain, straw and stover on one of the limed plots but not on the other (table 2).

Turning now to the effect of lime upon the soil itself, the chemical record shows (table 3) that the limed plots have lost considerably more carbon than the unlimed, for in 1917 the loss of carbon from plot 11B over plot 11A totaled 0.15 per cent of the soil weight, and from plot 24 over plot 21, 0.085 per cent. On the basis of 2,000,000 pounds of soil per acre this is a loss of 3000 pounds of carbon, or over 6000 pounds of organic matter in the one case and 1700 pounds of carbon, or over 3400 pounds of organic matter, in the second comparison, in which the rotation included legumes.

Timothy and clover were growing on the plots during the season (1916) when they were sampled for the tests given below. The limed soils were noticeably in the best physical condition, although lime had not been applied for the past five years. The vegetation looked best on plot 24, followed by 11B, 21 and 11A. Plot 11A had a very unfavorable appearance, being partly covered with patches of crab-grass. Its soil is unusually acid because of the annual applications of ammonium sulfate.

The above summary of the crop and soil history of these plots prepares the way for a better comprehension of the biochemical results obtained with the soil.

TABLE 3

Total carbon, total nitrogen and lime requirement of the plots in 1908, 1912 and 1917

PLOT NUMBER	TOTAL NITROGEN				TOTAL CARBON				LIME REQUIREMENT	
	1908	1912	1917	De-crease on limed plots	1908	1912	1917	De-crease on limed plots	1912	1917
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*
11A, unlimed.	}1118 {	0.0977	0.0904		}1.216 {	1.140	1.210		2100	2000
11B, limed...		0.0801	0.0819	0.0085		1.015	1.060	0.150	1500	1000
21, unlimed..	}1040 {	0.0771	0.0794		}1.130 {	1.030	1.170		1200	1200
24, limed		0.0810	0.0901	0.0107		0.995	1.085	0.085	Neu-tral	Alka-line

EXPERIMENTAL METHODS

Sufficient soil was obtained in November, 1916, from each of the four plots to permit the use of fresh-soil methods. These bulk samples were composites of 12 individuals from the surface 6 inches after about $\frac{1}{4}$ inch of the immediate surface had been scraped away. The samples were taken at a time when the soil was fairly dry and were sieved through a 5-mm. sieve, in the field, upon a piece of oilcloth and transferred to clean glass jars. Upon reaching the laboratory, portions of these samples were withdrawn for determinations of moisture and of the number of organisms. As soon as the moisture content had been ascertained, amounts equivalent to 200 gm. of oven-dry soil were weighed out into glass tumblers. Weighed amounts of organic matter were then added and mixed as thoroughly as possible with the soil. It is probable that the organic matter was not as well distributed as it would have been had the soil been dry. However, it seemed preferable to use the fresh, undried soil in order to approximate field conditions as nearly as possible. Finally, water was added to the extent of 50 per cent of the soil's maximum capacity. The tumblers were capped with petri dishes and incubated at 20° to 22°C. A similar set was used for the determinations of ammonia, nitrate and total nitrogen present at the beginning of the incubation periods.

Nitrates were determined by Allen's (1) reduction method, Devarda's alloy being used. Ammonia was determined by the magnesium oxide, and total nitrogen by the Kjeldahl method. Counts were made of the colonies appearing on Brown's egg albumim agar (2). The carbon-dioxide evolution was measured in an apparatus which has been previously used and described (6), except that the flat continuous base of the former apparatus was replaced by individual bases, grooved to receive the bell jars and sealed with paraffin (plate 1). Furthermore, the indrawn air was freed from carbon-dioxide by passing it through tubes containing $\frac{1}{4}$-mesh soda time (for carbon-dioxide determinations) instead of bubbling it through a potassium hydroxide solution. This caused the air pressure within the incubation chambers to be more nearly normal, which, together with a slow but continuous aspiration, made the conditions of the test approx'mate those existing in the fields.

The carbon-dioxide determinations on the samples taken in 1916 are not reliable because of leaks in the apparatus. Those reported are on samples taken in October, 1919.

It should be noted that the only materials added to the soil for these tests were: water, 0.75 gm. of soybean hay (seeds and stems) per tumbler for the carbon-dioxide determinations, dried blood sufficient to contain 100 mgm. of nitrogen for the ammonia and nitrate tests, and 1 per cent of mannite to the soil incubated for a determination of nitrogen fixation. The periods of incubation are given in the tables.

THE OXIDIZING POWER OF SOIL FROM THE LIMED AND UNLIMED FERTILITY PLOTS

The oxidation activities of the limed soils were greater than those of the unlimed soils throughout the 16-day period during which the evolved carbon dioxide was collected (table 4). The largest amount came from the limed

TABLE 4

The production of carbon dioxide obtained from mixing 0.75 gm. of soybean hay with 200 gm. of soil from limed and unlimed fertility plots

DAYS INCUBATED	PLOT 11A UNLIMED	PLOT 11B LIMED	PLOT 21 UNLIMED	PLOT 24 LIMED
	mgm.	*mgm.*	*mgm.*	*mgm.*
0–2	51.4	87.0	55.8	109.7
3–4	81.9	89.8	49.7	90.6
5–8	87.1	142.5	93.2	132.8
9–10	61.9	73.5	78.7	86.8
11–16	70.1	112.8	111.5	122.1
Total	352.4	505.6	388.9	542.0
Per cent increase over the unlimed plots.........	43.7		39.3	

soil of plot 24. This is the only one of the four soils which reacted alkaline to the Veitch test. The smallest amount was obtained from the soil of the unlimed plot 11A, which soil gave the greatest lime requirement by the Veitch

method. Comparing plots 11A and 11B which have had identical treatments, except in the case of liming, it is seen that the oxidizing power of the limed soil was 43.7 per cent greater than that of the unlimed. In a similar comparison the carbon-dioxide production was 39.3 per cent greater from soil of the limed plots than from that of the unlimed plot 21. Thus these data show that the greater the lime requirement of a Sassafras loam the smaller is its oxidizing power.

As these carbon-dioxide determinations are a direct measure of the rapidity of loss of organic matter from these plots, it is of importance to note the correlation between these measurements and the analyses for total carbon which have been made on the soil (table 3), by which Lipman and Blair have shown (3) that the adding of lime has caused a considerably increased depletion of the organic matter originally present in the soil 10 years ago. Moreover, the above carbon-dioxide tests reveal the present oxidizing power of the soils and indicate that if organic matter were added, in a very short time it would largely disappear from the limed plots. These tests were made with the soil two summers after lime had been applied and therefore represent an average oxidation activity, assuming that this activity was greater during the season that lime was applied than during the season four years later, before the next application of lime.

THE RELATION OF CARBON-DIOXIDE PRODUCTION TO OTHER MEASURES OF BIOCHEMICAL ACTIVITY

A measure of the ammonifying, nitrifying and nitrogen-fixing powers of a soil gives an indication of the nitrogen transformation possibilities therein, and merits consideration. A comparison of these factors with the carbon-dioxide production may be noted in table 5. Figure 1, which is based on this

TABLE 5

The production of carbon dioxide from soil of limed and unlimed plots as related to other biochemical factors

PLOT	CO₂ PRODUCTION IN 8 DAYS	NH₃-NITROGEN ACCUMULATION IN 6 DAYS	NO₃-NITROGEN ACCUMULATION IN 28 DAYS	NITROGEN FIXATION IN 20 DAYS	BACTERIAL NUMBERS	LIME TREATMENT
	mgm.	*mgm.*	*mgm.*	*mgm.*	*millions*	
11A	220.7	11.93	10.4	−0.02	2.5	None
11B	319.3	11.43	21.3	+1.35	6.2	2 tons per acre
21	198.7	11.37	16.1	−0.90	5.1	None
24	333.1	11.69	33.9	+7.00	6.5	2 tons per acre

table, shows that carbon dioxide-production, nitrate accumulation and bacterial numbers are considerably higher on the limed than on the unlimed plots. This is also true of the nitrogen-fixation values obtained but, although each of these values is the average of three or more determinations, a comparison

between the plots can not be made because the experimental error was in some cases greater than the differences found between treatments. The ammonia accumulation was nearly the same for all of the plots and did not correlate with the carbon-dioxide production.
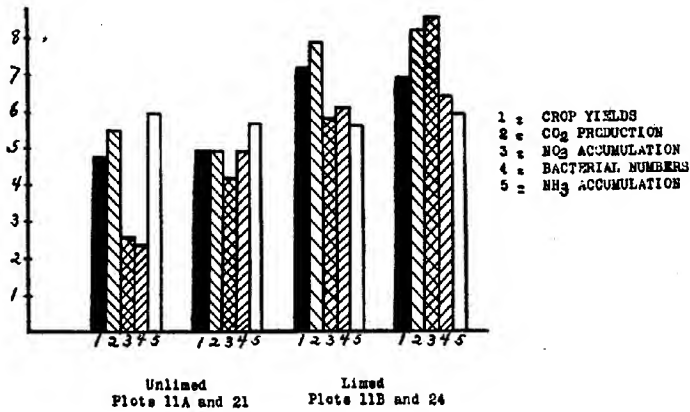
FIG. 1. A COMPARISON BETWEEN CROP YIELDS AND THE CO₂ PRODUCTION, BACTERIAL NUMBERS, NH₃ AND NO₃ ACCUMULATION OF SOIL FROM LIMED AND UNLIMED PLOTS

TABLE 6

Duplicate determinations of averages given in tables 4 and 5

PLOT NUMBER	CO₂ PRODUCTION	NH₃-NITROGEN ACCUMULATION	NO₃-NITROGEN ACCUMULATION	BACTERIAL NUMBERS	NITROGEN WITH MANNITE	FIXATION WITHOUT MANNITE
	mgm.	mgm.	mgm.	millions	mgm.	mgm.
11A	322.0	11.81	11.4	1.9	9.10	8.82
	382.7	12.05	9.2	3.1	8.82	8.54
				2.6	8.96	9.38
					8.68	
11B	477.1	11.32	20.2	5.8	10.08	8.12
	533.5	11.56	24.4	5.8	9.66	8.40
				6.9	9.24	8.40
21	419.6	11.93	14.0	4.5	7.84	7.84
	358.2	11.19	18.2	5.5	7.42	7.70
				5.3	7.84	7.84
24	552.5	11.32	33.0	6.6	8.82	8.26
	531.3	12.04	34.8	6.5	8.54	8.54
				Lost	8.40	8.40
					8.68	8.68

CROP PRODUCTION AS RELATED TO THE OXIDIZING AND NITRIFYING POWER
OF THE SOILS

The average increase in the dry weight of crops from the limed plots shows a marked relationship to the increased carbon-dioxide production and nitrate accumulation in the soil from the same plots. Bacterial numbers were also greater in the soil from which larger crops were obtained (fig. 1). But the relationship is especially close between crop yields and carbon-dioxide production. Thus the average increase in total dry matter on the limed plot 11B over the unlimed plot 11A is 28.3 per cent (table 2) and the increase in carbon dioxide production is 43.7 per cent. The corresponding increases for plot 24 over plot 21 were 32.0 and 39.3 per cent, respectively. When the percentage increase of crop production for the past 5 years is considered, it even more nearly approximates the percentage increase in carbon-dioxide production, as the differences between the crop-producing power of the plots were greater during the second rotation period.

CONCLUSIONS

If sufficient organic matter is present, the addition of basic substances, such as lime, generally results in a greater crop production and in a greater drain upon the soil. Assuming that the soil should not be allowed to become depleted in its supply of organic matter, the amount of liming to be done depends upon the balance between the cost of an increased feeding of the soil and the value of the increased crop production. Since plant growth is biological in nature, biological studies of a soil may possibly indicate its crop-producing possibilities more closely than purely chemical or physical studies— not considering fluctuating climatic conditions which are often the determining factors. This investigation gives evidence that future crop production may be indicated by a measure of the oxidizing and nitrifying power of the soil; and that some of the information as to what a soil needs in order to produce more, may be obtained from a manipulation of the same methods.

SUMMARY

1. Fresh soils from the surface 6 inches of four $\frac{1}{20}$-acre fertility plots at the New Jersey Agricultural Experiment Station were tested for their oxidizing, nitrifying, ammonifying and azofying powers.

2. The oxidizing power of the soil from the limed plots was approximately 40 per cent greater than that from the unlimed plots.

3. For this soil type (Sassafras loam) the oxidizing power varies inversely with its lime requirement.

4. Nitrate accumulation and bacterial numbers were higher on the limed soils whereas the ammonia accumulation was about the same for all of the plots.

5. The average crop yield for the past 10 years varies closely with the present oxidizing power of the soils. There is also a noticeable correlation between crop yield, nitrate accumulation and bacterial numbers, but not between crop yield and ammonia accumulation.

REFERENCES

(1) ALLEN, E. R. 1915 The determination of nitric nitrogen in soils. *In* Jour. Indus. Engin. Chem., v. 7, no. 6, p. 521–529.

(2) BROWN, P. E. 1912 Bacteria at different depths of some typical Iowa soils. Iowa Agr. Exp. Sta. Res. Bul. 11, p. 381–407.

(3) LIPMAN, J. G., AND BLAIR, A. W. 1913 Field experiments on the availability of nitrogenous fertilizers. N. J. Agr. Exp. Sta. Bul. 260.

(4) LIPMAN, J. G., AND BLAIR, A. W. 1919 Lime as a factor in soil fertility: I. Rotations without legumes. *In* Soc. Prom. Agr. Sci., Proc. 39th Ann. Meeting, p. 124–134.

(5) LIPMAN, J. G., BLAIR, A. W., McLEAN, H. C., AND MERRILL, L. F. 1913 Lime and rotation experiments. *In* N. J. Agr. Exp. Sta., 34th Ann. Rpt., p. 421–457.

(6) NELLER, J. R. 1918 Studies on the correlation between the production of carbon dioxide and the accumulation of ammonia by soil organisms. *In* Soil Sci., v. 5, p. 225–242.

PLATE 1

Fɪɢ. 1. Illustration showing four of the twelve units of an apparatus used for determining the carbon dioxide produced by soil organisms.
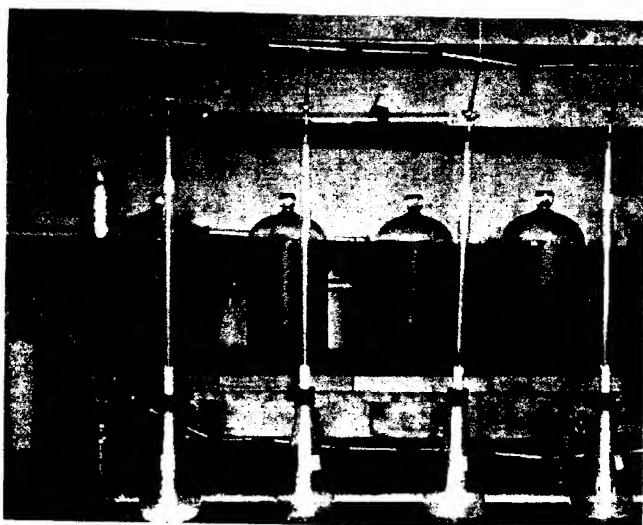
Fig. 1

THE DETERMINATION OF CARBON DIOXIDE IN WATER-INSOLUBLE CARBONATES[1]

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The methods described in this article are modifications of two recently devised for biological work, the original apparatus and methods of procedure having been altered to render them suitable for use with such materials as limestones, marls and soils. For purposes of discussion they will hereafter be designated as (a) the titrimetric method and (b) the gasometric method.

THE TITRIMETRIC METHOD

This method was originally devised primarily for the determination of the carbonate content of bones, though it was pointed out that it could also be used for any carbonate-containing material. The following is the technic originally described.

The sample is weighed into a tube 20 to 25 mm. in diameter and placed in a 250-cc. suction flask containing an excess of $0.1N$ barium hydroxide. A one-hole rubber stopper holding a dropping funnel is then inserted into the mouth of the flask in such a way that the stem of the funnel projects into and somewhat below the top of the tube containing the sample. A short piece of heavy-walled pressure tubing holding a screw clamp is slipped over the side arm of the flask. When this tube and funnel are in place the flask is evacuated to a pressure of less than 50 mm. of mercury and the screw clamp closed. Approximately $1 N$ hydrochloric acid is then allowed to enter slowly through the dropping funnel, running down the inside of the tube and decomposing the sample of carbonate with the liberation of carbon dioxide which is absorbed by the barium hydroxide. When decomposition is complete the flask is rotated for three minutes or more to break up the surface film of barium carbonate and permit the complete absorption of carbon dioxide, after which the screw clamp is opened and the stopper, funnel and tube removed, the liquid adhering to the outside of the tube being washed back into the flask. The excess barium hydrate in the flask after being washed through a Gooch crucible to separate it from the precipitated carbonate, is titrated with 0.1 N HCl, with phenolphthalein as the indicator.

This technic without modification is quite suitable for the analysis of lime-stones and marls. Certain precautions however, are to be observed. We found, for instance, that the violent evolution of gas taking place when the first drop of acid came into contact with the dry sample caused some of the solid to be carried either out of the tube entirely or so far up onto its walls that complete decomposition was attained only with difficulty. This was obviated by covering the sample with water. This diluted the first few drops of acid to such an extent that the initial reaction was slowed down so that the subsequent evolution of gas could be regulated.

As a matter of routine the addition of a drop or two of octyl alcohol is also to be advised. Unless this is done a persistent froth forms frequently with marls and occasionally with limestones which makes it necessary to add acid very slowly. This materially lengthens the time required to complete a determination.

The minimum amount of wash water must be used in transferring the excess barium hydroxide to the titration flask and this wash water must be neutral to phenolphthalein. The whole procedure should be carried out as speedily as possible with the minimum exposure of the barium hydrate to the air. To facilitate this we use a disc of filter paper in the Gooch crucible instead of the usual mat of asbestos. This allows the filtration to be carried out rapidly. The carbonate is precipitated in such a form that there is no danger of its passing through such a filter. The technic of the operation can be checked from time to time by blank determinations in which the whole procedure is carried out with the omission of the sample.

For the analysis of soils, which, as a rule, contain so little carbonate that it is necessary to take samples of several grams, the above procedure must be modified because of the difficulty in obtaining a complete mixture of the sample and the acid in a small tube nearly full of solid. We have therefore reversed the relative position of alkali and sample, weighing the sample directly into the flask from a weighing bottle and placing the standard alkali in the tube. The stem of the dropping funnel is bent slightly to, permit it to be placed in position *outside* of the tube and so admit the acid into the flask itself. In other respects the technic employed is the same as that followed above.

THE GASOMETRIC METHOD (1)

This method was originally designed for the determination of CO_2 in blood plasma but was suitable for the analysis of all carbonates in solution. We have so modified the apparatus that it can be used equally well for materials in the solid form, the only changes necessary being a larger burette to hold the greater volume of gas produced from samples of high-carbonate materials of usual size and an opening of sufficient diameter to permit the introduction of such samples. Figure 1 shows the apparatus.[2]

[2] The apparatus is being made by the Emil Greiner Company, 55 Fulton Street, New York.
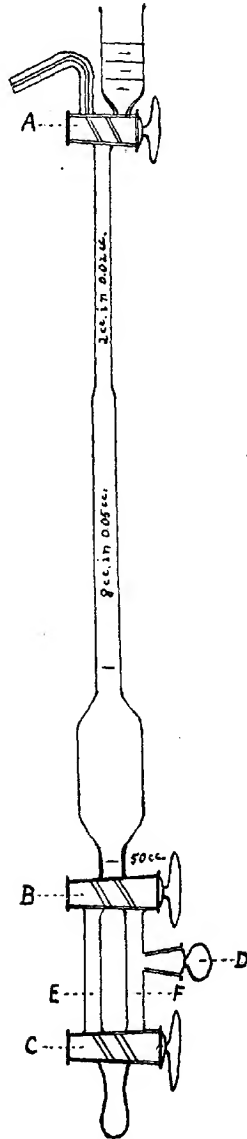
FIG. 1. APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE IN INSOLUBLE
OR UNDISSOLVED CARBONATES

It consists of a 10-cc. burette having the upper 2 cc. graduated in 0.02 cc. and the remaining 8 cc. graduated in 0.05 cc. The upper end of this burette is closed by a three-way stop-cock having one arm bent as illustrated and the other one sealed to a cup holding 5 to 10 cc. graduated to 5 cc. in 0.5 cc. The lower end of the burette is sealed to a bulb of such size that the whole apparatus will have a capacity of 50 cc. from the stop-cock A to a mark between the bulb and the stop-cock B. The openings in the stop-cocks B and C should be large, as mercury is forced through them.

The stopper D should be as close to B as convenient in order to reduce the space above it and the total capacity of the stopper and right-hand tube F to which the stopper is attached should be about 5 cc. The stopper should be hollow and the end should be open. It should be set at right angles to F.

The lower outlet of C is attached by a piece of heavy-walled suction tubing to a leveling bulb filled with mercury.

The following is the technic employed in making a determination. For marls, limestones or other materials so high in carbon dioxide that a sample of less than 500 mgm. will liberate not over 10 cc. of gas the hollow stopper serves as a weighing bottle and the material is weighed into it, the tube being filled with mercury up to the mouth of D to reduce the air space which must subsequently be evacuated. For carbonate-poor substances such as soils, the sample is weighed into the tube F, being introduced by means of a test-tube funnel.

The whole apparatus except the right-hand tube F between B and C (but including the right-hand hole in C) is then filled with mercury. With the stop-cock A closed and the connection open between B and C through the tube E, the leveling bulb is lowered to such a position that the mercury level drops below C, evacuating the burette, bulb, etc.[3] The cock B is then turned through a complete revolution establishing, as connection is made temporarily between them, equilibrium in gas pressure in the tube F and the evacuated space above it. The leveling bulb is then raised to a position above A and this cock opened, allowing the escape of the entrapped air. Repeating this operation several times reduces the air in the apparatus to a negligible amount. The sample is now held in a gas-free apparatus.

Approximately normal HCl is next poured into the cup above A and exactly 2.5 cc. admitted to the burette, the leveling bulb being held about even with the stop-cock B. The bulb is then lowered to the lowest position and the mercury allowed to flow out through the tube E, the cock B being closed while a little mercury still remains above it. If the sample is of such material that it is still contained in D, the mercury in the tube below it is now permitted to flow out through C, which is closed leaving a few drops above this cock to seal it. B is then turned to allow the acid to run into the tube F.

[3] This is conveniently done by means of a heavy cord of proper length attached to the bulb by one end and by the other to the support holding the apparatus.

The sample, if not originally weighed into the tube, is shaken out of the stopper into the acid. No precautions need be taken to moderate the violence of the reaction as any particles of the sample carried up into the bulb will be decomposed later. The apparatus should be shaken so that all of the sample is washed out of the stopper D and down from the walls of the tube.

TABLE 1

Carbon dioxide indicated by reading of V cc. of gas after a single extraction

TEMPERATURE OF ANALYSIS	AIR DISSOLVED IN 2.5 cc. H₂O SUBTRACT THIS FROM V AND MULTIPLY RESULT BY A TO CALCULATE MGM. CO₂	A	TEMPERATURE OF ANALYSIS	AIR DISSOLVED IN 2.5 cc. H₂O SUBTRACT THIS FROM V AND MULTIPLY RESULT BY A TO CALCULATE MGM. CO₂	A
°C.	cc.	mgm.	°C.	cc.	mgm.
15	0.051	$\frac{B}{760} \times 1.935$	23	0.045	$\frac{B}{760} \times 1.842$
16	0.050	$\frac{B}{760} \times 1.924$	24	0.044	$\frac{B}{760} \times 1.831$
17	0.049	$\frac{B}{760} \times 1.912$	25	0.043	$\frac{B}{760} \times 1.819$
18	0.048	$\frac{B}{760} \times 1.900$	26	0.042	$\frac{B}{760} \times 1.808$
19	0.048	$\frac{B}{760} \times 1.889$	27	0.041	$\frac{B}{760} \times 1.796$
20	0.047	$\frac{B}{760} \times 1.877$	28	0.040	$\frac{B}{760} \times 1.784$
21	0.046	$\frac{B}{760} \times 1.866$	29	0.040	$\frac{B}{760} \times 1.773$
22	0.045	$\frac{B}{760} \times 1.854$	30	0.039	$\frac{B}{760} \times 1.761$

TABLE 1A

Values of $\frac{B}{760}$

BAROMETER	$\frac{B}{760}$	BAROMETER	$\frac{B}{760}$	BAROMETER	$\frac{B}{760}$	BAROMETER	$\frac{B}{760}$
720	0.947	734	0.966	748	0.984	762	1.003
722	0.950	736	0.967	750	0.987	764	1.006
724	0.952	738	0.971	752	0.989	766	1.008
726	0.955	740	0.974	754	0.992	768	1.011
728	0.958	742	0.976	756	0.995	770	1.013
730	0.961	744	0.979	758	0.997	772	1.016
732	0.963	746	0.981	760	1.000	774	1.018

When the evolution of gas has stopped, the communication between the leveling bulb and F is opened and the stopper and tube completely filled with mercury up to the 50-cc. mark. B is then closed and the apparatus shaken with a rotary motion in such a way that the liquid is distributed in a thin

layer about the walls of the bulb until equilibrium between the gas in solution and that in the free space is attained. The liquid is next quickly drawn back into F, by lowering the leveling bulb and opening B which, however, is closed before any gas passes into it. C and B are finally turned to allow mercury to flow up into the burette through E while the acid is retained in F, the leveling bulb is raised until the mercury surface in it is on a level with that in the burette and the gas volume read. A fraction of a cubic centimeter of acid will unavoidably be held in the burette. This will cause no appreciable error in the results but care must be taken to read the gas volume at the surface of this liquid and not at the mercury surface, although it is the levels of the two mercury surfaces that are equalized.

The temperature and barometer readings should be noted at the time of reading the gas volume which affords sufficient data to permit the calculation of the weight of CO_2 obtained from the sample by means of the tables[4] 1 and

TABLE 2

Comparison of gasometric and titrimetric methods of determining carbon dioxide

SAMPLE	CARBON DIOXIDE		SAMPLE	CARBON DIOXIDE	
	Gasometric	Titrimetric		Gasometric	Titrimetric
	per cent	*per cent*		*per cent*	*per cent*
Calcium carbonate.......	43.53	43.84	Limestone, No. 4.	40.220	40.550
Limestone, No. 211......	43.32	43.21	Marl	42.400	42.290
Limestone, No. 212......	42.29	42.40	Soil, No. 5.............	0.015	0.014
Limestone, No. 213......	41.25	41.69	Soil, No. 10............	0.075	0.077
Limestone, No. 1........	43.88	43.97	Soil, No. 16............	5.000	4.860

1A. For a complete discussion of the principle of the method the reader is referred to the original article. Suffice it to say that it depends upon the generation of gas in a Torricellian vacuum, the measurement of that portion of the gas contained in a volume of 47.5 cc. in equilibrium with the gas dissolved in 2.5 cc. of water and the calculation of the total volume of gas from its known solubility in water at the temperature of the determination, correction being made for the air dissolved in the 2.5 cc. of water.[5]

Table 2 gives the results of analyses of various materials made by the two methods.

The deciding factor in the selection of the method to be used is the magnesium content of the material under examination. For magnesium-free or low-magnesium limestones the gasometric method is much to be preferred. As the magnesium content increases, the rate of decomposition decreases so

[4] Partly taken from Van Slyke (1, p. 317 and 360).
[5] This may however be determined for each analysis by introducing a few drops of alkali into the apparatus through the cup after reading the total volume of gas. The CO_2 will of course be absorbed leaving the air, the volume of which may then be read off after equalizing the mercury levels.

that with some samples of dolomite several hours are required for the complete decomposition of a 100 mgm.-sample. Under such circumstances the method of choice is, of course, the titrimetric one since the cost of the apparatus required for running several determinations simultaneously is small in comparison with that needed for making a corresponding number with the gasometric method.

In this laboratory the gasometric method, however, has proven of great value for the rapid, routine, proximate examination of marls and limestones where great accuracy is not required. For this purpose the reaction is allowed to proceed for several minutes until the initial violence has subsided. With pure calcium carbonate this time is sufficient for the completion of the reaction and the results will be correspondingly accurate. With ordinary, low-magnesium materials they will be low in proportion to the amount of magnesium present. Ordinarily, where this does not exceed 5 per cent the error will be less than 2 per cent.

Of the two methods the gasometric one is the more accurate. The possible sources of error in it are fewer and the gas volumes can be read to 0.02 cc., or about 0.04 mgm. CO_2. Assuming 0.05 cc. as the limit of accuracy for reading the titration values in the titrimetric method, the corresponding error would amount to approximately 0.1 mgm. CO_2. This value of course, is augmented by errors introduced in transferring solutions from one flask to another, the absorption of CO_2 from the air, etc.

REFERENCES

(1) VAN SLYKE, D. D. 1917 Studies of acidosis: Part II. Methods for determination of carbon dioxide and carbonates in solutions. *In* Jour. Biol. Chem., v, 30, p. 347.
(2) VAN SLYKE, D. D. 1918 Determination of carbon dioxide in carbonates. *In* Jour. Biol. Chem., v. 36, 351.

THE RELATION BETWEEN THE CALCIUM AND THE NITROGEN CONTENT OF PLANTS AND THE FUNCTION OF CALCIUM[1]

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The supply of calcium, especially in the carbonate form in the soil solution, becomes less as soils become more acid, and hence, in order to understand the relation of soil acidity to plant growth it is necessary to know something about the function of calcium in the metabolism of plants. One method of securing evidence regarding the function of an element is to observe the relations that exist between the amounts of this element and other elements or compounds that are present in different species of plants. In this way information may be obtained which indicates that either the element forms a part of certain compounds, or else it takes part in processes closely related to the formation or subsequent changes of the compounds. Both of these functions may, of course, be performed by an element. This brief preliminary paper sets forth the results obtained by applying this method of attack to the determination of the function of calcium, and reports a fairly close relationship between the calcium and the nitrogen content of plants, indicating that at least to some extent the use of nitrogen by plants involves the use of calcium.

THE RELATIVE COMPOSITION OF PLANTS

In table 1 the composition of 34 species of plants is given with respect to nitrogen, calcium, phosphorus, magnesium, and potassium. These data were taken from the sources indicated, and are believed to be as reliable as any available. In the case of some plants the results of analyses of the different parts were found recorded without the weights of these parts. When this was the case estimates of the percentage of the total weight formed by the different parts were made and used in the calculation of the composition of the plant as a whole. The percentages of magnesium, phosphorus, and potassium were obtained from the sources indicated for calcium.

Figure 1 gives a graphic representation of the data of table 1 and is better for comparative purposes. The plants are arranged from left to right in the order of increasing percentages of nitrogen.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

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TABLE 1

The composition of plants (dry-matter basis)

NUMBER	KIND OF PLANT	CHARACTER OF MATERIAL ANALYZED	NITROGEN			CALCIUM			RELATIVE AMOUNTS		RATIO OF CALCIUM TO NITROGEN	PER CENT		
			Number of analyses	Reported by	Per cent	Number of analyses	Reported by	Per cent	N	Ca		Magnesium	Phosphorus	Potassium
1	Sorghum (*Sorghum vulgare, saccharatum*)	Fodder	94	5	0.96	4	12	0.41	1.0	1.0	0.431	0.15	0.16	1.40
2	Meadow Fescue (*Festuca pratensis*)	Hay	21	5	1.23	2	2	0.44	1.0	1.0	0.360	0.11	0.16	1.78
3	Water Grass (*Paspalum laeve*)	Hay	1	12	1.30	1	12	0.43	1.0	1.0	0.330	0.21	0.17	1.33
4	Red-Top (*Agrostis vulgaris*)	Hay	40	5	1.30	4	2	0.43	1.0	1.0	0.330	0.10	0.17	0.91
5	Timothy (*Phleum pratense*)	Hay	3	12	1.36	3	12	0.41	1.0	1.0	0.305	0.13	0.32	1.91
6	Corn (*Zea mays*)	Fodder	708	5	1.39	7	12	0.60	1.0	1.5	0.427	0.39	0.26	1.74
7	Orchard Grass (*Dactylis glomerata*)	Hay	46	5	1.42	4	2	0.38	1.0	1.0	0.252	0.20	0.20	1.66
8	Kentucky Blue Grass (*Poa pratensis*)	Hay	26	5	1.53	4	12	0.30	1.0	1.0	0.196	0.10	0.22	1.78
9	Oats (*Avena sativa*)	Fodder	24	5	1.62	9	*	0.38	1.5	1.0	0.234	0.14	0.30	2.67
10	Potatoes (*Solanum tuberosum*)	Ripe	1	12	1.73	*	12	0.55	1.5	1.5	0.318	0.30	0.29	1.74
11	Rye (*Secale cereale*)	Hay	38	5	1.92	1	12	0.38	2.0	1.0	0.197	0.17	0.17	1.91
12	Bermuda Grass (*Cynodon dactylon*)	Hay	2	12	1.98	2	12	0.55	2.0	1.5	0.268	0.16	0.43	3.15
13	Barley (*Hordeum sativum*)	Fodder	1	12	1.99	1	12	0.69	2.0	2.0	0.348	0.09	0.32	2.67
14	Wheat (*Triticum vulgare*)	Fodder	15	5	2.11	2	12	0.34	2.0	1.0	0.163	0.15	0.34	2.90
15	Horse Radish (*Cochleria armoracia*)	Mature	1	12	2.13	1	12	0.71	2.0	2.0	0.335	0.39	0.23	2.07
16	Sugar Beet (*Beta vulgaris*)	Mature	86	12	2.23	†	12	0.65	2.5	2.0	0.295	0.24	0.30	0.75
17	Lupine (*Lupinus hirsitus*)	Half ripe	2	5	2.28	2	12	0.97	2.5	2.5	0.423	0.18	0.33	1.78
18	Onion (*Allium cepa*)	Mature	1	12	2.29	2	12	1.42	2.5	3.5	0.621	0.45	0.29	1.78
19	Red Clover (*Trifolium pratense*)	In bloom	36	5	2.38	1	12	1.72	2.5	4.0	0.721	0.36	0.21	1.08
20	Alsike Clover (*Trifolium hybridum*)	In bloom	5	5	2.40	113	12	1.16	2.5	3.0	0.482			
21	Sweet Clover (*Melilotus alba*)	Hay	18	5	2.53	3	2	1.39	3.0	3.5	0.548			
22	Mammoth Clover (*Trifolium medium*)	In bloom	7	5	2.56	3	12	1.49	3.0	3.5	0.583	0.58	0.22	2.57
23	Serradella (*Ornithopus sativa*)	In bloom	1	12	2.59	1	12	1.39	3.0	3.5	0.538	0.19	0.43	2.65

24	Radish (Raphanus sativus)..........	Mature	1	2.64	12	1	12	1.50	3.0	3.5	0.568	0.28	0.33	2.49
25	White Clover (Trifolium repens).......	Green	1	2.64	12	1	12	1.32	3.0	3.5	0.501	0.35	0.37	2.49
26	Cauliflower (Brassica oleracea, botrytis).......	Mature	1	2.72	12	1	12	1.15	3.0	3.0	0.423	0.35	0.26	2.98
27	Crimson Clover (Trifolium incarnatum)....	In bloom	22	2.76	5	4	12	1.37	3.0	3.5	0.497	0.22	0.19	1.16
28	Rape (Brassica napus)...........	In bloom	37	2.78	5	6	12	1.24	3.0	3.0	0.447	0.19	0.43	2.15
29	Soybean (Glycine hispida)..........	Hay	23	2.80	5	2	4	1.96	3.0	5.0	0.718	0.74	0.32	0.91
30	Vetch, common (Vicia sativa)........	Green	14	2.98	5	25	12	1.39	3.5	3.5	0.467	0.32	0.33	1.91
31	Cowpea (Vigna unguiculata)........	Hay	35	3.39	5	1	4	1.93	4.0	4.5	0.570	0.45	0.24	0.83
32	Tobacco (Nicotiana tabacum)........	Ripe	1	3.47	12	1	12	2.69	4.5	5.0	0.766	0.48	0.42	3.06
33	Lettuce (Lactuca sativa)..........	Green	3	3.94	12	3	12	1.90	5.0	4.5	0.481	0.68	0.72	
34	Cabbage (Brassica oleracea, capitata).......	Mature	2	4.02	12	2	12	1.90	5.0	4.5	0.472	0.36	0.53	

* 59 analyses of tubers, 6 of tops.
† 149 analyses of roots, 25 of tops.
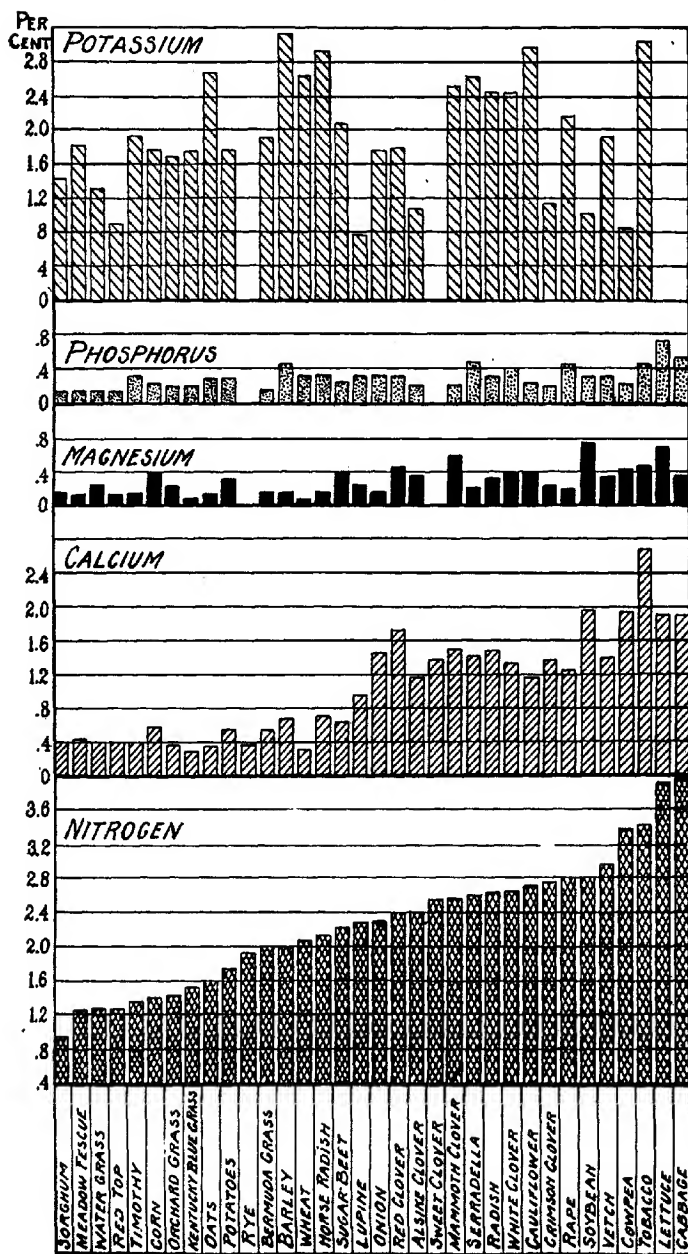
FIG. 1. DIAGRAM SHOWING THE RELATION BETWEEN THE AMOUNTS OF NITROGEN, CALCIUM, PHOSPHORUS, MAGNESIUM, AND POTASSIUM IN THE PLANTS INDICATED

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The outstanding feature of this figure is the relation between the calcium and the nitrogen content of the plants. The potassium content varies considerably, and there is no relation between it and the nitrogen content. Phosphorus increases with nitrogen, but to a much smaller extent. Phosphorus is not a constituent of all proteins, and therefore it need not necessarily vary with the nitrogen content. Furthermore, a considerable portion of the phosphorus usually exists in an inorganic form. According to the work of Ames and Boltz (1) approximately 50 per cent of the total phosphorus in the alfalfa plant is in an inorganic form. Hence, while there should be some relation between the nitrogen and phosphorus content, it need not necessarily be very close.

The magnesium content is more irregular and is relatively low in all cases. Its function being probably that of a phosphorus carrier, one would expect it to vary with the phosphorus and nitrogen, as it does in a very general way. Since the same magnesium may possibly be used over again for this purpose the total amount present need not necessarily increase regularly with increasing amounts of nitrogen and phosphorus.

THE RELATION OF CALCIUM TO NITROGEN

Calcium increases more regularly with increasing nitrogen than any of the other elements. There are marked variations, but since the data were taken from many sources, the results may not be strictly comparable in all cases. However, the data do indicate that there is a relation between the calcium and the nitrogen content of plants. For ease of comparison and in order to

TABLE 2

Range of nitrogen and calcium percentages according to relative figure

RELATIVE FIGURE	RANGE OF NITROGEN PERCENTAGES DESIGNATED BY RELATIVE FIGURE	RANGE OF CALCIUM PERCENTAGES DESIGNATED BY RELATIVE FIGURE
1.0	0 −1.30	0 −0.48
1.5	1.31–1.60	0.49–0.68
2.0	1.61–1.90	0.69–0.88
2.5	1.91–2.20	0.89–1.08
3.0	2.21–2.50	1.09–1.28
3.5	2.51–2.80	1.29–1.48
4.0	2.81–3.10	1.49–1.68
4.5	3.11–3.40	1.69–1.88
5.0	3.41 and above	1.89 and above

eliminate small irregularities due to various causes, relative figures of the nitrogen and calcium contents of the plants are included on the basis indicated in table 2.

The agreement of the relative figures is fairly good. In the case of plants high in nitrogen, the relative figures for calcium are usually higher than for

nitrogen. This is due to a higher ratio of calcium to nitrogen as indicated in the table. The ratios of calcium to nitrogen for the first seventeen plants as given in the table, are of the same order. The ratios of the last seventeen plants are of another order which is nearly twice as large as that of the first group. This indicates that the plants of the last group require nearly twice as much calcium for a given nitrogen content as do those of the first group. This relation is clearly shown in figure 1.

The plants are thus conveniently divided into two groups. The first group, having the average calcium-nitrogen ratio of 0.306 is composed almost entirely of members of the grass family, plants which as a class have a low lime requirement (11) and are quite tolerant to soil acidity. The second group, having an average ratio of 0.553, includes the legumes and plants which in most cases respond to liming and are sensitive to soil acidity. These data indicate an important difference in the metabolic processes of the two groups. Just what this difference is has not been determined definitely, but a possibility is indicated in the following discussion.

FUNCTION OF CALCIUM

Calcium functions in the plant in at least two ways. It serves as plant-food material and as such enters into the composition of proteins and other plant substances (8). In many plants, especially those high in calcium, a relatively small portion of the total amount is required for this purpose. A greater portion of the calcium taken up by the plant is probably used for the neutralization and precipitation of the acids in the plant sap (11). The carbonate and bicarbonate are the principal forms found in the soil solution that will perform the latter function. These forms after entering the plant react with the acids neutralizing them, liberating carbon dioxide. Oxalic acid is one of the strongest and more common of the plant acids. Reacting with calcium bicarbonate, it forms the neutral and insoluble calcium oxalate. Crystals of this oxalate are found in many plants.

The sources of plant acids are not definitely known. Many metabolic processes within the plant undoubtedly give rise to acids, some of which may be viewed as by-products. Some investigators hold that carbohydrate metabolism is an important source of plant acids. Protein formation involves reactions in which considerable amounts of acid may be produced. The decomposition of proteins in the life processes of plants offers many other possibilities for acid formation. The decomposition is probably brought about by oxidation, and as has been indicated by several investigators (10), the oxidation of protein produces acids among which are acetic, succinic, capronic, formic and oxalic.

Since a large portion of the calcium in many plants is used for the neutralization of acids, there should be more calcium in plants producing large amounts of acid. Assuming protein metabolism as an important source of

acids, a high protein or nitrogen content should be accompanied by a high calcium content. This conforms with the data presented in table 1.

As already indicated, the group of plants with a high calcium and a high nitrogen content also has a higher ratio of calcium to nitrogen. This may be partly due to the existence of poorer conditions for the complete oxidation and destruction of acids in this group, and hence the greater need and use of calcium carbonate for the neutralization of the acids. Since the conditions for oxidation in the different plants undoubtedly vary, an explanation is offered for the marked individual differences in the calcium-nitrogen ratio of certain plants. This explanation conforms with the suggestion of Mac-Dougal, Richards, and Spoehr (9) that acid formation and accumulation in some plants may be due to poor oxidizing conditions within the plant tissue. On this basis, poor conditions for oxidation in plants increase the need for calcium in the carbonate form. There are, of course, a large number of other possible factors that may affect this need, and hence the ratio of calcium to nitrogen: e.g., the varying amounts of nitrogen in other than protein form may affect the calcium-nitrogen ratio to an appreciable extent in some cases.

The relation of calcium to nitrogen and important plant compounds and processes needs to be more carefully investigated with the more refined methods which have been devised in recent years. Undoubtedly, if a large number of different plants were grown with different soil treatments and then analyzed at various stages for calcium and different constituents and conditions, much extremely valuable information would be obtained regarding the relation of these to plants. In this connection the publications of Burd and Hoagland regarding some of these relations should be mentioned (3, 6, 7).

SUMMARY

1. There is a rather close relation between the calcium and nitrogen content of plants.

2. The contents of potassium, phosphorus and magnesium do not bear this close relation to the nitrogen content.

3. The important agricultural plants may be divided into two groups; viz., (a) those having a low calcium-nitrogen ratio and a low lime requirement, and (b) those having a high calcium-nitrogen ratio and a higher lime requirement.

4. Protein metabolism is probably one of the chief sources of plant acids and this may give rise to the need of calcium in the carbonate form for the neutralization of these acids.

REFERENCES

(1) AMES, J. W., AND BOLTZ, G. E.   1912   Nitrogen and the mineral constituents of the alfalfa plant.   Ohio Agr. Exp. Sta. Bul. 247.

(2) BEAL, W. H.   1889   Compilations of the analyses of fodder articles, fruits, sugar-producing plants, dairy products, etc.   *In* Mass. State Agr. Exp. Sta. 7th Ann. Rpt., p. 291–313.

(3) BURD, J. S.   1919   Rate of absorption of soil constituents at successive stages of plant growth.   *In* Jour. Agr. Res., v. 18, no. 2, p. 51–72.

(4) CROCKER, C. S.   1893   Compilations of the analyses of fodder articles, fruits, sugar-producing plants, dairy products, etc.   *In* Mass. State Agr. Exp. Sta. 10th Ann. Rpt., p. 309–336.   •

(5) HENRY, W. A., AND MORRISON, F. B.   1916   Feeds and Feeding, p. 633–646.   The Henry-Morrison Co., Madison, Wis.

(6) HOAGLAND, D. R.   1919   Relation of nutrient solution to composition and reaction of cell sap of barley.   *In* Bot. Gaz., v. 68, no. 4, p. 297–304.

(7) HOAGLAND, D. R.   1919   Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant.   *In* Jour. Agr. Res., v. 18, no. 2, p. 73–117.

(8) LOEW, O.   1903   The physiological rôle of mineral nutrients in plants.   U. S. Dept. Agr. Bur. Plant Indus. Bul. 45.

(9) MACDOUGAL, D. T., RICHARDS, H. M., AND SPOEHR, H. A.   1919   The basis of succulence in plants.   *In* Bot. Gaz., v. 67, p. 405–416.

(10) MANN, G.   1906   The Chemistry of Proteins, p. 237–249.   Macmillan Co., New York.

(11) TRUOG, E.   1918   Soil acidity: I. Its relation to the growth of plants.   *In* Soil Sci., v. 5, p. 169–195.

(12) WOLFF, E.   1870 and 1880   Aschen Analysen, pt. 1, 1870, 194 p., pt. 2, 1880, 170 p., Wiegant and Hempe, Berlin.

THE EFFECT OF DICALCIUM SILICATE ON AN ACID SOIL[1]

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At the meeting of the American Chemical Society at Boston in 1917 Cowles (1), and Scheidt presented experimental data with plants from which they concluded that dicalcium silicate has a greater value than either ground limestone or calcium hydrate as a fertilizer material, and that silicon is an essential element and promotes plant growth.[2]

Upon the solicitation of Mr. Cowles, who desired to have the work repeated with other soils and under other conditions, advantage was taken of an opportunity afforded in connection with an experiment conducted for another purpose, to include "dicalcium silicate" and "hydrated silica," prepared by the Electric Smelting and Aluminum Company, Sewaren, N. J., of which Mr. Cowles is president.

According to Mr. Cowles, 75 to 80 per cent of the dicalcium silicate may be considered as having the composition of $(CaO)_2SiO_2$, the remainder being zeolitic material composed of sodium, calcium, aluminum and silicon which has resisted the solvent action of boiling water for an hour. The following analyses accompanied the material:

	per cent
SiO_2	28.70
Al_2O_3	6.27
Fe_2O_3	1.88
CaO	46.81
MgO	2.94
CO_2 and H_2O	6.93
Na_2O	6.47
	100.00

It is the leached residue of a sinter produced from a mixture of silicates, lime and sodium carbonate in the manufacture of disodium aluminate.

The hydrated silica was reported as having the following composition:

	per cent
SiO_2	55.90
H_2O	40.01
Na_2SO_4	4.09
	100.00

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[1] Contribution 271 from the Rhode Island Agricultural Experiment Station at Kingston.
[2] Results published by the Electric Smelting and Aluminum Company, Sewaren, N. J.

The Miami silt loam which was used in the pot experiment involving these materials was taken near the Rhode Island Agricultural Experiment Station plats and had been in turf for many years without manurial treatment. Fifteen pounds of the soil containing 18 per cent moisture were used in each 8-inch Wagner pot.

The alkaline materials and the largest applications of acid phosphate were added on May 6, 1919, so that they could react with the soil prior to the addition to each pot, on May 24, of the following basal fertilizer:

<div align="right">grams</div>

Nitrate of soda	1.5
Sulfate of potash and magnesia	5.0
Acid phosphate	3.5

On July 5, 1 gm. each of nitrate of soda and sulfate of potash was also added.

Cos lettuce was planted on May 26, but in some cases more seed had to be added on June 6. On July 3, final thinning was made to 6 plants per pot. Where plants of the first seeding had to be supplemented by those of the second seeding a somewhat uneven growth resulted, and the parallelism from duplicate pots was less satisfactory than it would have been had there been always the same proportions of plants from the first and second seeding. It is believed, however, that conclusions are warranted, even though the results are not closely quantitative. The crop was harvested on July 22.

The special applications in addition to the basal fertilizer, and the yields resulting from them, are given in table 1.

It may be seen that where only the basal application of fertilizer was applied without any other additions (pair 1), the lettuce made only a very small growth. Pairs 9, 10 and 11 show that maximum growth was attained by different proportions of calcium carbonate and acid phosphate, and even by a very large application of acid phosphate (pair 13). If desired to estimate the applications on an acre basis, it may be observed that 7.25 gm. per pot is equivalent to 1 ton per acre. It was shown by other pots that the basal fertilizer supplied enough for nutrient purposes.

Concerning an investigation described by the authors in another paper (2) the following statement was made at the close: "The results indicate that the practical advantage of phosphating and liming may often prove to be due to the precipitation of active aluminum quite as much as to supplying phosphorus as a nutrient, and lime as a reducer of acidity."

By comparing pairs 2 and 3 it appears that the dicalcium silicate corrected the condition about equally with an equivalent amount of limestone. Full opportunity was given for each to exert its maximum effect, as shown by the fact that in pair 4, 50 per cent additional limestone increased the growth.

There were no indications that the silicon in the dicalcium silicate was of any value. The same is true of the silicon in the hydrated silica, as may be seen by comparing pair 5 without—and pair 6 with—hydrated silica; or again,

pair 7 without—and pair 8 with—hydrated silica. The smaller yield in one of pair 7 seems to be attributable to the fact that five of the six plants were the partially developed ones of the second planting previously referred to.

The dicalcium silicate was as effective as limestone in counteracting the toxic conditions existing in the acid soil, but there was no evidence that there is any justification in claiming an additional value because of its content of silicon.

TABLE 1

Yields of dry cos lettuce leaves from duplicate pots, with the extra applications in addition to the basal fertilizer

PAIR	EXTRA APPLICATIONS	YIELDS OF DRY LETTUCE LEAVES
		gm.
1	None	3.0 4.5
2	Dicalcium silicate, 20.29 gm	12.0 13.0
3	Limestone, 20 gm., equivalent to the above	11.5 12.0
4	Limestone, 30 gm	15.0 18.5
5	Limestone, 5 gm	9.0 11.0
6	Limestone, 5 gm.; hydrated silica, 7.25 gm. (1 ton per acre)	7.0 10.0
7	Limestone. 5 gm.; acid phosphate, 16.5 gm	9.0 18.0
8	Limestone, 5 gm.; acid phosphate, 16.5 gm.; hydrated silica, 7.25 gm	19.5 20.0
9	Limestone, 5 gm.; acid phosphate, 26.5 gm	20.0 21.0
10	Limestone, 10 gm.; acid phosphate, 11.5 gm	19.0 19.0
11	Limestone, 15 gm	8.5 10.0
12	Limestone, 15 gm.; acid phosphate, 6.5 gm	17.0 19.0
13	Acid phosphate, 100 gm	21.0 23.0

Even if it should ever be proved that, contrary to the opinion now generally held, silicon is an essential element, it seems probable that there would be enough of it active in our siliceous soils to satisfy all needs.

It should occasion no surprise if silicon were to have an advantageous indirect effect under conditions which were not shown to be optimum. If such an effect were observed, however, it would, obviously, be no proof that silicon is essential to plant growth.

REFERENCES

(1) COWLES, ALFRED H. 1917 Calcium silicates as fertilizers. *In* Metal. Chem. Engin., v. 17, p. 664–665.

(2) HARTWELL, BURT L., AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *In* Soil Sci., v. 6, p. 259–277.

MISCELLANEOUS SOIL INSECTICIDE TESTS

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The practical control of insects which spend the whole or a greater part of their lives underground has been in the past and continues to be a most difficult problem. In America we have depended almost entirely on agricultural practices for the control of soil-inhabiting insects, particularly those attacking field crops. Some years ago apparently successful experiments were conducted against white grubs in lawns with kerosene emulsion (1) but subsequent tests by other entomologists were so conflicting that the use of kerosene emulsion has never become general, although frequently advocated in literature.

Carbon bisulfide has been recommended for the grape *Phylloxera*, grub worms, and other underground insects in Europe and especially in France, and has been suggested by writers in America, but like kerosene emulsion it has never come into general use. More recently (8) carbon bisulfide has been found to be quite effective for destroying the woolly aphis and data at hand indicate its practical usefulness against other underground pests. Its use for general crop pests such as white grubs seems impractical, however, as was illustrated in a series of experiments conducted by W. H. W. Komp, a senior student at Rutgers College and working under the direction of Dr. Thos. J. Headlee (6). He found that common white grubs (*Lachnosterna* sp.) in lawns could be controlled by injecting carbon bisulfide into the soil at the rate of approximtely ¾ ounce to the square foot which would cost, at 10 cents a pound, $272 per acre, an amount prohibitive except for very intensively cropped land or very small areas. These experiments were not sufficiently comprehensive for conclusive results and, furthermore, the bisulfide was injected to a depth of only 3 inches, which condition would probably not permit a maximum diffusion of the gas. Our own experience with carbon bisulfide indicates that its use against most white grubs and similar insects is impractical, but that it can be used to advantage to destroy ant colonies and to kill grubs which have an open burrow, such as grubs of the southern green June beetle (*Cotinis nitida*).

More recently the cyanides—sodium cyanide, potassium cyanide, and hydrocyanic acid—have come into prominence as effective and practical for the control of many underground pests. The pioneer work with sodium cyanide as a soil insecticide should probably be attributed to J. A. Hyslop

of the United States Bureau of Entomology and F. A. Kaufmann of the
Roessler and Hasslacher Chemical Company. Hyslop's experiments in 1913
(7) showed that commercial sodium cyanide (39 to 40 per cent cyanogen)
applied dry in hills of corn or potatoes at the rate of 300 pounds per acre, will
kill wireworms and not injure the soil permanently but that it cannot be
applied with safety to crops while the crops are on the land or immediately
prior to seeding. Although Kaufmann's results were not recorded in litera-
ture, as early as October 1915 he issued a mimeographed statement of his
experiments which was distributed generally to entomologists, and his results
were the basis for trying out cyanide against the Japanese beetle grub. In
this mimeographed statement Kaufmann records the effective use of granular
sodium cyanide in solution to destroy ants and white grubs (presumably
Lachnosterna sp.) at a strength which will not ordinarily injure grass. The
strength advised is $\frac{1}{8}$ ounce of sodium cyanide to 1 gallon of water, this amount
to be sprinkled over an area of about 6 square feet ($56\frac{11}{16}$ pounds in 7260
gallons of water per acre) which is afterwards thoroughly watered.

A carefully planned series of experiments were made by Peterson (10) for
the control of wireworms with sodium cyanide and his results show that
while these insects can be killed with large quantities of cyanide the amount
necessary (300 pounds per acre) to bring about control makes it too expensive
for ordinary use. Within the past few months, results of experiments with
sodium cyanide for the control of the peach borer have been published (2).
In this case the writer concludes from his experiments that this material is
unreliable because, on account of its solubility under varying conditions, it
often proved injurious to trees. The experiments are not reported in detail
. but one might infer that under certain prescribed conditions cyanide could
be used effectively and without injury to peach trees.

Experiments conducted in 1914 and 1915 with calcium cyanamide (45
per cent $CaCN_2$) against the root-knot nematode by J. R. Watson led him to
conclude (11) that this material applied at the rate of from 1 to 3 tons per
acre and thoroughly mixed with the soil reduced the number of nematodes
to an extent sufficient to permit profitable growing of susceptible plants.
Injurious effects of the cyanide to newly planted crops persisted for a number
of months in some cases. Sodium cyanide was found to be a satisfactory
control for this nematode according to the experiments conducted and re-
ported by W. P. Duruz (4). He reports "nearly a perfect control" in green-
houses when two applications were made at the rate of 200 pounds of sodium
cyanide in 14,520 gallons of water per acre for each application. Moist warm
soil and aeration by occasional stirrings were necessary for the best results.
Seeds of such plants as tomato, radish and cucumber germinated satisfactorily
when planted a week after the last treatment. Another series of experi-
ments recently recorded by L. P. Byars (3) shows that sodium cyanide (2
parts) and ammonium sulfate (3 parts) applied at the rate of 3600 and 5400
pounds per acre failed to eradicate completely the root-knot nematode and

the author concludes that the method is not practical against this nematode although it seemed to be more efficient than most chemicals which have been tried. There seemed to be no difference in effectiveness when applied dry and as a liquid.

Comprehensive and valuable data on cyanide gas as a soil fumigant have been obtained and reported by E. R. de Ong (9). His experiments were conducted to determine the effect on plants, diffusion in soil, etc., rather than to determine the effect on insects and with applications in gaseous form rather than in liquid. His most important conclusions from our point of view were that "a heavy damp or a very wet sandy soil is almost impervious to hydrocyanic-acid gas" and that "gas generated in a soil body diffuses with extreme slowness in clay soils or very wet sandy soils, but in sand with a medium amount of moisture, diffusion of gas is much more rapid." He concludes that "sodium cyanide offers a satisfactory means of fumigating masses of loose, porous soil, especially those with only small amounts of clay, or of seed-beds and potting soil" and that "such treatments allow of much wider range of concentrations when the soil is not occupied by a crop."

The author wishes to review here his own brief experience with the more common soil insecticides.

KEROSENE EMULSION

Kerosene emulsion has been repeatedly recommended for various kinds of underground insects but very few experiments have been made to affirm or disprove its value. In 1888 W. B. Alwood tested kerosene emulsion against

TABLE 1

Results with kerosene emulsion against Popillia grubs, Riverton, N. J.

CHARACTER OF FIELD	RATE PER ACRE			AREA TREATED	AREA EXAMINED	DATE TREATED	DATE EXAMINED	RESULTS		KILL	DATE REEXAMINED	RESULTS		KILL
	Kerosene emulsion	Amount per acre	Additional water					Dead	Alive			Dead	Alive	
	per cent	gal.		sq. ft.	sq. ft.	1919	1919			per cent	1919			per cent
Thin weeds { and grass {	8	10,450	None	100	9	Sept. 15	Sept. 22	48	76	38.7+	Sept. 25	26	20	54.3+
	4	20,900	None	100	9	Sept. 15	Sept. 22	45	77	36.8+	Sept. 25	15	32	31.9+
Timothy { stubble .. {	8	10 450	None	100	9	Sept. 18	Sept. 22	1	3	25.0	Sept. 23	2	4	33.3+
	4	20,900	None	100	9	Sept. 18	Sept. 22	3	13	18.7+	Sept. 23	0	14	0

the white grub of the southern green June beetle (*Cotinis nitida*) on the Capitol grounds at Washington, D. C., with apparent success. However, his statements are contradictory to the life history of this species of grub and it therefore appears likely that other grubs were present and confused his conclusions. A few years ago the writer had an opportunity to test out kerosene

emulsion against grubs of *Cotinis* on a golf green at Louisville, Ky. An 8 to 10 per cent emulsion was found to be quite effective in destroying 80 per cent of these grubs when applied in August at the rate of 1 gallon to 6 or 8 square feet (5445 to 7260 gallons per acre) and afterwards throughly washed in with water. A slight browning of the tips of the grass was the only injury to the grass when the treated area was afterwards sprinkled with water. Against grubs of the green Japanese beetle (*Popillia japonica*) we obtained a kill of 25 to 54 per cent (table 1) when an 8 per cent kerosene emulsion was used at the rate of 1 gallon to 4 square feet (10,890 gallons per acre). In the same series of tests against *Popillia* grubs sodium cyanide has always given an appreciable better kill than the emulsion. We conclude that, as a rule, kerosene emulsion is not as satisfactory a soil insecticide as is cyanide because it is less effective, more expensive and more difficult to make up and apply.

COAL TAR OR CREOSOTE PREPARATIONS

Emulsifiable coal tar or creosote preparations which are essentially composed of coal-tar oils, 53 per cent (percentages approximate); phenols, 12 per cent; water 10 per cent; and a saponifying agent, 25 per cent, are little known as soil insecticides. Our own experience with "Carco," a commercial preparation, and Barrett's disinfectant (sold only for disinfecting purposes but analyzing about the same as "Carco," giving equal insecticide results and very much cheaper) has not been extensive but we did find that it killed grubs of the green June beetle, being only slightly less effective than kerosene emulsion when diluted 1 to 125 parts of water and applied the same as the emulsion, that is, 1 gallon of diluted mixture to 6 or 8 square feet and afterwards sprinkled with water. The creosote mixtures brought a large percentage of the green June beetle grubs to the surface where they died; but kerosene emulsion is apparently a more rapid killing agent, as the dead grubs are more often in the soil although a few do come onto the surface before they die. We have repeatedly tested Barrett's disinfectant against the green Japanese beetle grub and the recorded results are given in table 2.

As a soil insecticide against the commoner white grubs it appears to be equal to kerosene emulsion but not as good as sodium cyanide.

MISCELLANEOUS TESTS

Corrosive sublimate. Repeated tests have proven the ineffectiveness of corrosive sublimate against our common white grubs.

Sulfuric acid. According to reports sulfuric acid has been used with apparent success against white grubs, in Europe. We tested it against the Japanese beetle grub, using it at the strength of 1 of acid to 96 of water, 1 to 48, and 1 to 24, applying 2616, 1308 and 872 gallons of diluted solution, respectively, per acre, but with none of these applications could we get better than a 10 per cent kill.

TABLE 2

Results with Barrett's disinfectant against Popillia grubs, Riverton, N.J.

CHARACTER OF FIELD	RATE PER ACRE			AREA TREATED	AREA EXAMINED	DATE OF TREATMENT	DATE OF EXAMINATION	RESULTS		KILL
	Barrett's disinfectant	Water	Additional water					Dead	Alive	
	gal.	gal.		sq. ft.	sq. ft.	1919	1919			per cent
1. Timothy and bluegrass	54.5	2616	Yes	100	9	Oct. 15	Oct. 20	15	26	36.6
2. Timothy and bluegrass	54.5	5232	Yes	100	9	Oct. 15	Oct. 20	8	11	42.1+
3. Timothy and bluegrass	54.5	7848	Yes	100	9	Oct. 15	Oct. 20	22	37	37.3–
4. Timothy and bluegrass	54.5	5232	No	100	9	Oct. 22	Oct. 27	9	15	37.5
5. Timothy and bluegrass	81.7	5232	No	100	9	Oct. 22	Oct. 27	8	6	57.1+
6. Timothy and bluegrass	54.5	5232	Yes	100	9	Oct. 22	Oct. 27	6	10	37.5
7. Timothy and bluegrass	54.5	5232	No	100	9	Oct. 29		5	2	71.4+
8. Timothy and bluegrass	81.7	5232	No	100	9	Oct. 29		3	3	50.0
9. Timothy and bluegrass	54.5	5232	Yes	100	9	Oct. 29		7	0	100.0
10. Bluegrass lawn	54.5	5232	Yes	100	4	Oct. 30	Nov. 3	29	35	45.3+
11. Heavy bluegrass	54.5	6976	Yes	100	4	Oct. 31	Nov. 4	1	5	16.7–
12. Heavy bluegrass	81.7	6976	Yes	100	4	Oct. 31	Nov. 4	1	6	14.3–

Note: "Carco" was used in Nos. 11 and 12. Depth of grubs at this season and heaviness of sod accounts for the low per cent of mortality. In plot 10, 51 of the grubs were Cyclocephala and 13 Popillia. None of the latter and 56.8+ per cent of the former were killed by the treatment. All of the Popillia grubs were 4 to 6 inches below the surface at the time of treatment.

Acetaldehyde was used against the green Japanese beetle grub in varying strengths up to 1 to 48 of water at the rate of 5232 gallons of diluted solution to the acre, and 1 to 96 at the same rate per acre. It was wholly ineffective in a number of tests and the very best kill was less than 12 per cent.

Kopper's solution. A by-product received from the Kopper's Company and reported to analyze approximately 25 per cent carbon bisulfide and 75 per cent benzene gave negative results when used at a dilution of 1 to 48 and 5232 gallons of diluted solution to the acre, the best kill of a number of tests being 9 per cent.

SODIUM CYANIDE

Among the important considerations in determining methods of controlling the Japanese beetle were methods of destroying the insect in the grub stage since the greater part of its life is as a grub in the ground. Mr. Goodwin, at the time in charge of the control operations, conducted numerous experiments, as reported at the last annual meeting of the American Association of Economic Entomologists (5), obtaining a kill of 65 to 90 per cent of the grubs where sodium cyanide was used in solution at the rate of approximately 110 pounds in 26,000 gallons of water, per acre. The past spring a considerable area infested by the grubs was treated according to this formula, but later counts showed that the kill averaged not over 25 per cent for the season. Experiments conducted by the use of a hand sprinkler gave far better results and a careful study of the conditions clearly indicated that the low mortality was due, not so much to the material used as to the method of application which in this case did not allow sufficient penetration. Accordingly, the sprinkler pipes for use the past fall were modified and as a result the cyaniding operations, which have been in charge of Mr. C. H. Hadley, on the 32 acres treated have given an average kill of about 80 per cent, the average up to the latter part of October being about 90 per cent but rapidly dropping off as the cold weather set in. Granular sodium cyanide was used at the rate of 165 pounds in 12,000 gallons per acre. Plate 1 illustrates the two types of sprinklers referred to above, the one (fig. 1) having 1-inch holes 10 inches apart, while the other (fig. 2) has $\frac{3}{8}$-inch holes averaging 48 holes to the foot, the pipe in both cases being 3-inch.

Two methods of applying the liquid sodium cyanide were used. For small-plot tests of one to several hundred square feet the ordinary sprinkling can, with the holes enlarged to about $\frac{3}{32}$-inch in diameter, was employed. This method is applicable for small areas such as lawns and home gardens. For large areas of one-half to ten or twenty acres 600-gallon tanks mounted on heavy wagon frames and drawn by a caterpillar-type tractor were used. These were fitted with perforated pipes as described above and illustrated in plate 1, figure 2. The flow was by gravity and regulated by a gate valve which could be operated by the driver of the tractor. The water supply was obtained from nearby creeks by means of a centrifugal pump and pipe line

Results with sodium cyanide against Popillia grubs, tank applications, Riverton, N. J.

CHARACTER OF FIELD	RATE PER ACRE			AREA TREATED	NUMBER EXAMINED	DATE TREATED	DATE EXAMINED	RESULTS		KILL
	NaCN	Water	Additional water			1919	1919	Dead	Alive	
	lbs.	gal.	gal.	acres	sq. yd.					per cent
1. Robert's timothy stubble	220	8,000	None	0.3	3	Sept. 16	Sept. 20	30	1	96.7+
2. Robert's timothy stubble	220	Dry	8,000	0.3	3	Sept. 17	Sept. 20	16	15	51.6+
3. Robert's timothy stubble	165	8,000	None	0.3	3	Sept. 16	Sept. 20	41	1	97.6+
4. Robert's timothy stubble	165	Dry	8,000	0.3	3	Sept. 18	Sept. 20	18	9	66.6+
5. Robert's timothy stubble	110	8,000	None	0.2	3	Sept. 13	Sept. 20	16	2	88.8+
6. Bailey's hay stubble	165	12,000	None	11.0	16	Oct. 7	Oct. 9	86	45	65.6+
7. Jessup's old hay stubble	165	11,000	None	3.0	10	Oct. 20–21	Oct. 24	240	44	84.5+
8. Jessup's new hay stubble	165	11,000	None	2.0	12	Oct. 21–22	Oct. 25	165	12	93.2+
9. Robert's rye stubble	165	11,000	None	4.0	9	Oct. 6–9	Oct. 9	6	5	54.5+
10. Bailey's hay stubble	110	12,000	None	0.1	3	Sept. 21	Sept. 25	16	5	76.1+
11. Bailey's hay stubble	165	12,000	None	0.1	3	Sept. 21	Sept. 25	29	5	85.2+
12. Bailey's hay stubble	220	12,000	None	0.1	3	Sept. 21	Sept. 25	59	2	96.7+
13. H. Lippincott pasture	110	12,000	Watered	0.1	3	Oct. 30	Nov. 4	10	2	83.3+
14. H. Lippincott pasture	110	12,000	Rain	0.1	3	Oct. 30	Nov. 4	12	0	100.0
15. H. Lippincott pasture	165	12,000	Rain	0.1	3	Oct. 30	Nov. 4	11	2	84.6+
16. H. Lippincott pasture	165	12,000	Rain	0.1	3	Oct. 30	Nov. 4	3	0	100.0
17. H. Lippincott pasture	110	12,000	Watered	0.1	3	Nov. 4	Nov. 7	24	9	72.7+
18. H. Lippincott pasture	165	12,000	None	0.1	3	Nov. 4	Nov. 7	20	4	83.3+
19. H. Lippincott pasture	110	12,000	Watered	0.1	3	Nov. 4	Nov. 7	20	6	76.9+
20. H. Lippincott pasture	165	12,000	None	9.0	6	Oct. 30–Nov. 7	Nov. 13, 18	46	41	52.8+
21. H. Lippincott pasture	165	12,000	None	0.5	5	Nov. 7–10	Nov. 17	10	16	38.4+

In plot 7 one square yard examined gave 6 dead and 30 live grubs. This examination was near the edge of the field and because of the low kill in this particular instance the percentage of mortality was appreciably lowered, the per cent of kill without this count being 94.3+.

Low mortality in plot 9 can be accounted for by the matted condition of the wild growth, preventing thorough penetration.

In plots 13 to 21 there were a few Lachnosterna grubs and in a few cases these were not distinguished from Popillia grubs. In plot 13 six of the grubs were Lachnosterna, all of which were dead. In plot 17, seven were Lachnosterna, 85.7+ per cent of which were dead and in plots 18 and 19, four and one, respectively, were Lachnosterna, all of which were dead.

In plot 20 only the areas treated November 6 and 7 were examined. The grubs were 3 inches or more below the surface and the temperature of the soil 48°F. or lower.

TABLE 4

Results with sodium cyanide against Popillia grubs, sprinkler applications, Riverton, N. J.

CHARACTER OF FIELD	RATE PER ACRE			AREA TREATED	NUMBER EXAMINED	DATE TREATED	DATE EXAMINED	RESULTS		KILL
	NaCN	Water	Additional water					Dead	Alive	
	lbs.	gal.	gal.	sq.ft.	sq.ft.	1919	1919			per cent
Weeds—thin weeds	110	Dry	16,000	100	9	Sept. 15	Sept. 19	108	37	74+
	165	Dry	16,000	100	9	Sept. 15	Sept. 19	95	24	79+
	220	Dry	16,000	100	9	Sept. 15	Sept. 19	96	2	97+
	110	16,000	None	100	9	Sept. 16	Sept. 19	85	12	88+
	165	16,000	None	100	9	Sept. 16	Sept. 19	88	4	95+
	220	16,000	None	100	9	Sept. 16	Sept. 19	95.	3	97—
	Check						Sept. 19	0	42	0
	110	Dry	16,000	100	9	Sept. 18	Sept. 22	6	20	23+
	165	Dry	16,000	100	9	Sept. 18	Sept. 22	5	10	33+
	220	Dry	16,000	100	9	Sept. 18	Sept. 22	6	1	85+
	110	16,000	None	100	9	Sept. 18	Sept. 22	7	0	100
	165	16,000	None	100	9	Sept. 18	Sept. 22	3	0	100
	220	16,000	None	100	9	Sept. 18	Sept. 22	19	0	100
	Check						Sept. 22	0	84	0
Campbell's heavy timothy	164	6,976	None	100	9	Oct. 15	Oct. 20	21	19	52+
	56¾	6,976	None	100	9	Oct. 15	Oct. 20	34	22	61—
	113	6,976	None	100	9	Oct. 15	Oct. 20	35	15	70
	57	6,976.	Watered	100	9	Oct. 22	Oct. 27	17	6	74+
	57	6,976	None	100	9	Oct. 22	Oct. 27	10	8	55+
	85	6,976	Watered	100	9	Oct. 22	Oct. 27	21	11	66—
	85	6,976	None	100	9	Oct. 22	Oct. 27	23	7	77—
	113½	6,976	Watered	100	9	Oct. 22	Oct. 27	18	4	82—
	113½	6,976	None	100	9	Oct. 22	Oct. 27	16	5	76+
	170¾	6,976	Watered	100	9	Oct. 22	Oct. 27	12	2	86—
	170¾	6,976	None	100	9	Oct. 22		14	0	100
	57	6,976	None	100	9	Oct. 29		13	4	76+
	57	6,976	Watered	100	9	Oct. 29		4	2	67+

Campbell's heavy timothy	85	6,976	None	100	9	Oct. 29		17	3	85
	85	6,976	Watered	100	9	Oct. 29		3	2	60
	113½	6,976	None	100	9	Oct. 29		15	1	94—
	113½	9,676	Watered	100	9	Oct. 29		9	1	90
	170½	6,976	None	100	9	Oct. 29		12	0	100
	170½	6,976	Watered	100	9	Oct. 29		13	1	93—
Woods—heavy bluegrass	75⅜	4,650	Heavy Watered	100	9	Oct. 31	Nov. 4	15	12[1]	55+
	38	4,650	Heavy Watered	100	9	Oct. 31	Nov. 4	9	16[4]	36
	45⅞	5,580	Heavy Watered	100	9	Oct. 31	Nov. 4	6	14[1]	30
	57	6,976	Heavy Watered	100	9	Oct. 31	Nov. 4	3	2[1]	60
Riverton lawn	57	1,308	Watered	100	3	Oct. 4	Oct. 8	51[2]	0	100
	113½	2,614	Watered	100	3	Oct. 4	Oct. 8	29[2]	0	100
	164½	3,920	Watered	100	3	Oct. 4	Oct. 8	40[2]	0	100
Cinnaminson lawn	45⅜	5,568	Watered	100	4	Oct. 30	Nov. 3	26[3]	4	87—
	57	6,968	Watered	100	4	Oct. 30	Nov. 3	45[4]	16	74—
	38	4,646	Watered	100	4	Oct. 30	Nov. 3	71[4]	12	85+
Burlington lawn	57	6,976	Watered	100	4½	Oct. 16	Nov. 20	41[8]	0	100
	113½	3,924	Watered	100	4½	Oct. 16	Nov. 20	119[7]	0	100
	170½	3,924	Watered	100	1	Oct. 16	Nov. 20	26[8]	0	100

[1] Live grubs 5 to 6 inches deep.
[2] All Cyclocephala grubs except one which was Lachnosterna.
[3] Eighteen Cyclocephala—all dead.
[4] Forty Cyclocephala—90 per cent dead.
[5] Seventy-six Cyclocephala—85 per cent dead.
[6] Twenty-four Cyclocephala and 17 Macrodactylus.
[7] One hundred and six Cyclocephala and 13 Macrodactylus.
[8] Eighteen Cyclocephala and 8 Macrodactylus.

and where a field of several acres was being treated it has been found most economical to use three tanks, two tractors and three men, one to mix and the other two to drive the tractors. As one tank was emptied the driver returned to the water-supply pipe, there leaving the empty tank to be filled and returning to the field with a full tank. The empty tank was filled by the time the other tank in the field was emptied and thus the cyaniding operations were continuous with no loss of time for tractors or men. By this method 3 tanks can cover 3 acres in a day. Where it is possible to approach the water pipe from both sides one stand-pipe is sufficient but where it is necessary to fill along roadways it is necessary to place two stand-pipes, to prevent blocking the road as illustrated in plate 2, figure 1. By this method and by using granular sodium cyanide (96 to 98 per cent cyanogen) at 165 pounds per acre the cost per acre for material (cyanide $26\frac{1}{2}$ to $30\frac{1}{2}$ cents) and labor ($4.00 to $5.00 per day) was $49.00 to $56.50

Dry cyanide was applied by broadcasting for small plots and with a fertilizer drill for larger plots. The latter method is not entirely satisfactory on account of clogging, especially in moist weather.

The results of a series of experiments given in tables 3 and 4 are worth recording. The tests and examinations were made by men familiar with our methods of applying the insecticide and with methods of making examinations but not otherwise experienced in entomological practices. The experiments are somewhat miscellaneous in nature, as it was impossible to follow up all details on account of pressure of other work and the services of an experienced experimenter were not available.

In obtaining the results, areas 1 by 9 feet were dug at more or less regular intervals in the treated field. An eye hoe was used for this purpose and the loosened soil (pl. 2, fig. 2) was then carefully examined by hand to determine the number of dead and live grubs. Where the plots were small individual square feet instead of square yards were examined.

In general, our observations clearly indicate that the percentages of mortality as given in tables 3 and 4 are low, since the grubs, especially the smaller ones, disintegrated rapidly after death and were easily overlooked. It was found that ground covered with timothy, weeds or similar vegetation permitted better penetration of the insecticide if the crop was closely mowed and consequently a more satisfactory kill was obtained than where the vegetation was tall or matted. Applications of dry cyanide, broadcasted or drilled, and the treated area afterwards watered gave appreciably and uniformly poorer results than where the cyanide was applied in liquid form. Where comparisons were possible we observed that cyanide was more effective against *Cyclocephala*, *Lachnosterna*, and *Macrodactylus* grubs than against *Popillia;* in some cases the difference was apparently due to greater resistance and in some cases because the *Popillia* grubs were earlier influenced by approaching cold weather and had penetrated deeper during the latter part of October than had the other grubs.

Little is known of the effect of cyanide treatment on soil. Byers (3) reports no serious injurious or retarding effect on the germination or growth of dasheens where sodium cyanide had been applied, but that small quantities increased the growth and larger treatments retarded growth the first season; others report similarly. In our own experiments it was found that grass might be burned by the cyanide solution but that there was no permanent injurious effect except where the liquid stood in low places for a considerable period of time. Cultivated crops such as corn were appreciably injured by the treatment. We have no definite observations on the permanent effect of cyanide treatment on the soil but we do know, from chemical analyses made by C. S. Cathcart, state chemist of New Jersey, that the cyanide disappears in the course of a week or ten days after treatment or even in a shorter period in case it rained during the interval. J. C. Clark, of the Henry A. Dreer Company, used soil treated with sodium cyanide (1 and 2 ounces per 100 pounds) and sodium cyanide with ammonium sulfate (2 ounces NaCN and 3 ounces $(NH_4)_2SO_4$ per 100 pounds of soil) for several varieties of seedling ferns and found no serious injurious after-effect, except to certain sensitive varieties, particularly the holly fern which died when started in soil previously treated as above. Walter S. Lenk, of the Roessler and Hasslacher Chemical Company, has kindly furnished us with numerous reports of experiments and observations made by F. A. Kaufmann and R. N. Sargent, which give further light in the use and value of sodium cyanide as a soil insecticide. Their results indicate the value of a combination of granular sodium cyanide and ammonium sulfate (400 pounds NaCN and 500 to 600 pounds $(NH_4)_2SO_4$ per acre) the two being added to the soil successively and thoroughly mixed. The addition of ammonium sulfate accelerates the decomposition of cyanide and not only gives a prompt reaction but is at the same time a fertilizer of some value. They found that soil thus treated could be safely planted with most crops one to two weeks after treatment. We have had no personal experience with sodium cyanide in combination with ammonium sulfate. Before cyanide can be recommended for use in sterilizing greenhouse soil many more experiments will be necessary to determine the chemical effect on soil treated and the effect on different kinds of greenhouse plants when such soil is used for potting and seed-beds.

It is evident for the foregoing remarks that while many isolated experiments have been conducted to determine the possible use of sodium cyanide as a soil insecticide, the whole study, which is a most important one, lacks continuity and until a consistent and continuous program of investigation is vigorously inaugurated by some institution, preferably the United States Department of Agriculture, we cannot look for other than fragmentary results and temporary conclusions.

JOHN J. DAVIS

REFERENCES

(1) ALWOOD, W. B. 1888 Kerosene emulsion as a remedy for white grubs. *In* U. S.
      Dept. Agr., Insect Life, v. 1, p. 48–50.
(2) BLAKESLEE, E. B. 1919 Use of toxic gases as a possible means of control of the
      peach-tree borer. U. S. Dept. Agr. Bul. 796.
(3) BYARS, L. P. 1919 Experiments on the control of the root-knot nematode, *Hetero-
      dera radicicola* (Greef) Mueller: I. The use of hydrocyanic acid gas in loam soil
      in the field. *In* Phytopathology, v. 9, p. 193–204.
(4) DURUZ, WILLIS P. 1917 A study of the root nematode (*Heterodera radicicola*) and
      its control. *In* Soil Sci., v. 4, p. 481–492.
(5) GOODWIN, W. H. 1919 Japanese flower beetle. *In* Jour. Econ. Ent., v. 12, p. 247–
      252.
(6) HEADLEE, T. J. 1915 White grub remedies. *In* N. J. Agr. Exp. Sta. 36th Ann.
      Rpt., p. 318–320.
(7) HYSLOP, J. A. 1914 Soil Fumigation, v. 7, p. 305–312.
(8) LEACH, B. R. 1918 Experiments in the control of the root form of the woolly apple
      aphis. *In* U. S. Dept. Agr. Bul. 730, p. 29–40.
(9) DE ONG, E. RALPH 1917 Hydrocyanic acid gas as a soil fumigant. *In* Jour. Agr.
      Res., v. 11, p. 421–436.
(10) PETERSON, ALVAH 1917 Laboratory and field experiments with sodium cyanide
      and other chemicals against wireworms in the soil. *In* N. J. Agr. Exp. Sta.,
      38th Ann. Rpt. p. 469–479.
(11) WATSON, J. R. 1917 Control of root-knot by calcium cyanamide. Fla. Agr. Exp.
      Sta. Bul. 136.

PLATE 1

FIG. 1. Cyaniding tank, illustrating improper application resulting when a few large holes
are used in the distributor pipe.

FIG. 2. Uniform distribution and better penetration is secured when the holes in the
distributor pipes of the cyaniding outfit are smaller and numerous.
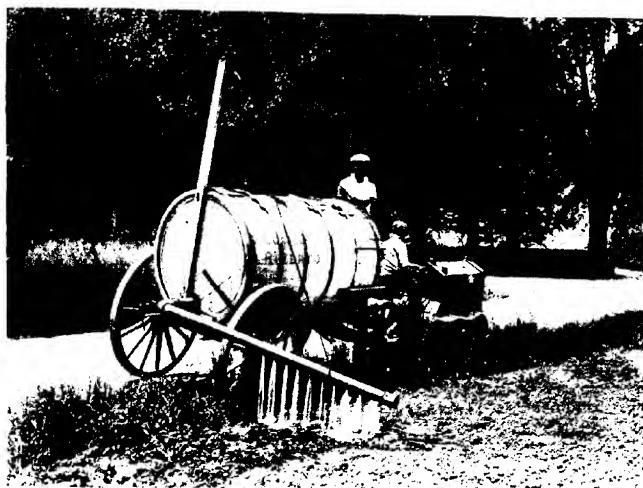
FIG. 1
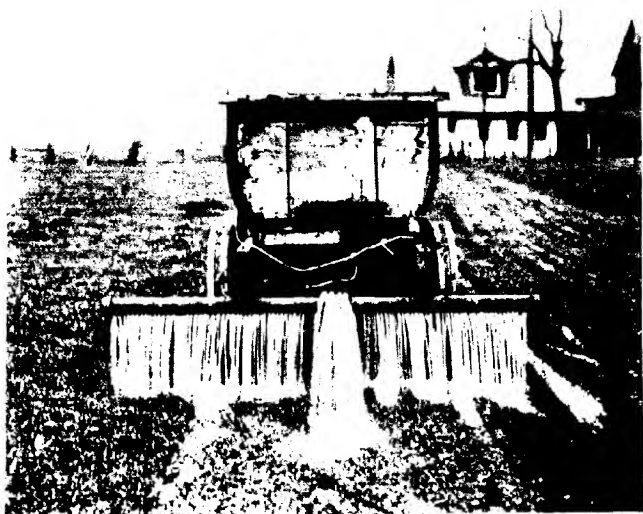
FIG. 2

PLATE 2

Fig. 1. Filling station for large-scale cyaniding operations.
Fig. 2. Examining soil to determine effect of soil insecticide treatments.

FIG. 1

FIG. 2

THE ANTAGONISTIC ACTION OF CALCIUM AND IRON SALTS TOWARD OTHER SALTS AS MEASURED BY AMMONIFICATION AND NITRIFICATION

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Salts which may occur in soils and those applied to them in various operations influence the physical, chemical and biological nature of the soil. The changed physical and chemical nature may modify the number, species, and physiology of the soil microflora. Hence, the question arises as to what effect a certain fertilizer, soil amendment or soil alkali is going to have and especially how it acts upon the bacterial flora of the soil. This last question is very complex and vital. Its answer necessitates a knowledge of the direct and the indirect reactions into which the chemical enters, also a knowledge of the complex reactions catalyzed by it. Yet these are vital, for the reclaiming of alkali soils and the maintaining of others in a productive condition advances as our knowledge of the intricate changes which the various salts produce within a soil become greater.

Papers have already been published on the relative toxicity of various salts found in or applied to a soil as measured in terms of ammonification (4) and nitrification (5), also upon the stimulating influence of various salts on bacterial activities and the manner in which the stimulation is exerted (6).

It is the purpose of this paper to consider the antitoxic action of calcium and iron salts toward other compounds. Calcium and iron compounds were selected because (a) they exert a marked effect upon plant growth; (b) they influence very materially the physical and chemical properties of the soil; (c) calcium sulfate is used rather extensively in the reclaiming of black alkali land, and (d) they are abundant and comparatively cheap; hence, if found effective they may be used in reclaiming soil which contains moderate quantities of alkalies.

A careful review of the literature has been made. That dealing with the first two phases of the problem has been summarized in the earlier publications (4, 5, 6). It is, therefore, necessary to consider in this paper only briefly the literature dealing with the antagonistic action of salt toward salts.

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[1] Most of the analytical work of this study was done by Messrs. Goldthorpe, Carter, and Poulter.

HISTORICAL

Although it has been understood for some time that animals and plants require a balanced nutrient for proper development, yet the modern conception of antagonism is due to Loeb who began publishing in 1901. A general account of his work appears in his "Dynamics of Living Matter" (10). He worked on the development of *Fundulus* eggs in various solutions and found that these eggs are unable to form an embryo if put immediately after fertilization into solution of pure sodium chloride of the same concentration as that in sea-water. This toxicity is reduced if a small definite quantity of the salt of a bivalent metal is added to the sodium chloride. The salt of any bivalent metal is able to produce this effect except those of very poisonous metals, such as mercury. Even such poisonous salts as those of lead are able to produce this result. That this effect is due to the cation was shown by using different salts of the same metals. Trivalent cations were also capable of rendering the toxicity of salts of univalent metals less harmful; but a tetravalent cation, thorium, was found to have only a slight antitoxic effect. The reverse is also true, for monovalent ions were found capable of reducing the toxicity of salts of zinc. A slight antagonism was observed between bivalent cations, such as strontium and magnesium.

The relative quantity of the antitoxic ion that has to be added varies with the concentrations of the toxic solution. Thus with *Fundulus* a concentration of 0.25 M sodium chloride is harmless. In a 0.625 M sodium chloride solution one bivalent ion is required to render 1,000 sodium ions harmless. It was found impossible to render harmless a solution of sodium chloride above a certain concentration.

It is interesting to note that these antagonistic effects hold only for the eggs and not for the larvae of *Fundulus*. Loeb, therefore, concludes that the antagonistic effects appear only so long as the fish is surrounded by the egg membrane and they are actually due to the two kinds of ions mutually hindering one another's diffusion into the egg.

The work of Loeb on animals has been continued and developed by Osterhout with much ingenuity with regard to plants. His work and that of his contemporaries has been summarized by Atkins (1), Robertson (6), Stiles and Jorgensen (7). The writer, therefore, has no need of making a review of the literature on this subject but will consider only briefly some of the general conclusions which have been reached.

Before ideas on antagonism had taken a definite form Loew (8, 9,) elaborated a theory to account for the toxic properties of magnesium when calcium was not present in sufficient quantity. He supposed calcium to be a necessary constituent of the chlorophyll bodies and nucleus, and when magnesium is present in great excess this takes the place in those bodies that should be occupied by calcium. This causes a structural disturbance on account of which the protein substances cease to be active and death ensues. It will

be observed that this will only explain the need for a balance between calcium and magnesium and is not a general theory of antagonism. In the case of calcium and magnesium the seat of the antagonistic action, according to this theory, is inside the cell in the nucleus and plastids, whereas Osterhout (16) considers that the value of calcium lies in its effect at the surface between the absorbing membrane and the external solution, and is not due to chemical action inside the cell. He, therefore, explains antagonism by assuming that antagonistic substances prevent each other from entering the cell. A difficulty which has been urged against it—that substances slowly penetrate into the cell wall even in a properly balanced solution—he does not consider vital if it is supposed that the antagonistic substances affect certain life processes which control permeability. So long as they are present in the right proportions their effect on these processes is favorable and their penetration into the cell can do no harm. The preservation of normal permeability is regarded as the result rather than the cause of antagonism.

Pauli (17) regards the plasma-membrane as a carrier of ions into the interior of the cell. The plasma-membrane is supposed to form compounds with the ions and by the reversibility of the process the ions enter the cell. From this, Szucs (22) formulates the theory that if there is outside the cell a mixture of salts containing two different ions, both of which are carried in by the same radical of the plasma-membrane, these ions must naturally hinder one another's absorption; each will combine with a part of the plasma-membrane substance which would otherwise be used by the other ion if that alone were present, and so the absorption of both ions is hindered.

Out of the work has grown the idea of physiologically balanced solutions, or as Osterhout conceives it, "normal life is possible only when the necessary salts combine with the colloids of the living substance in a definite ratio."

This conception of antagonism and balanced solutions was first applied to a study of bacteria by Lipman in 1909. In his (11) experiments on the rate of ammonification of *Bacillus subtilis* he showed that there is some antagonism between sodium and magnesium. On the other hand, he (12) found no antagonism but increasing toxicity when magnesium and calcium were combined. Later he (13) demonstrated that there exists, as measured by ammonification, a true antagonism between sodium chloride and sodium carbonate, and between sodium sulfate and sodium carbonate, thus indicating that the cations as well as the anions may at times play a part in antagonism.

Kelley (7), in studying the ammonification and nitrification of certain soils, found no antagonism between magnesium and sodium. However, Lipman and Burgess (14) observed in the case of nitrogen fixation by *Azotobacter chroococcum* an antagonism between sodium and magnesium.

Winslow and Falk (23) have observed antagonistic effects in experiments on *Bacillus coli*. They found that cultures suspended in solutions of sodium chloride or calcium chloride were decreased in number; that higher concentrations produced sterilization of the culture; and that a combination of

sodium chloride and calcium chloride in the molecular proportions of 5 to 1 was favorable to the growth of the organisms.

Shearer (19, 20) has also demonstrated similar effects of salts upon the viability of *Meningococcus* and *Bacillus coli*. He found that a combination of sodium chloride and calcium chloride was favorable to growth, whereas each salt used separately produced a decrease in growth.

Brooks (2) found that, as measured by the rate of respiration of *Bacillus subtilis*, there is a marked antagonism between sodium chloride and calcium chloride, and between potassium chloride and calcium chloride. The antagonism between sodium chloride and potassium chloride is slight, and the antagonism curve shows two maxima.

Later Brooks (3), using the same method and organism, found a well-marked antagonism between magnesium chloride and sodium chloride, and, contrary to the findings of Lipman (12), a very slight antagonism between magnesium and calcium.

EXPERIMENTAL WORK

The soil used in this work, taken from the College Farm, at Logan, Utah, is of a sedimentary nature. It was deposited by streams flowing into the valley, laden with débris derived from the nearby mountains, which are composed largely of quartzite and limestone. A physical and chemical analysis of the soil is given in table 1.

The soil used, therefore, was a sandy loam very high in acid-soluble constituents, but the water-soluble constituents were not excessive. The calcium and magnesium contents were very high and mainly in the form of the carbonate. The soil was well supplied with phosphorus and potassium, and there was a fairly large quantity of iron present. In fact, all of the elements of plant-food were present in abundance, with the exception of nitrogen, which was low. The soil was very productive, and previous work had shown the ammonifying and nitrifying powers of the soil to be about the average for the soils of the arid regions. The nitrogen-fixing powers of the soil were above the average, and previous work had shown it to have an intensely interesting bacterial flora.

Several hundred pounds of the soil were thoroughly mixed, stored in a large box, and kept as near field conditions as possible so that all the work could be done on the same soil. As the soil was needed in the work, portions were brought to the laboratory, air-dried in the dark, then weighed in 100-gm. portions into sterile covered tumblers. To each of these was added 2 gm. of dried blood. The whole was then carefully mixed, and the salt in most cases added from a carefully standardized stock solution. This, together with sufficient sterile distilled water to make the moisture content up to 20 per cent, was thoroughly mixed in the soil. Each series, together with sterile blanks, was incubated at 28° to 30°C. and analyzed for ammonia or nitrates as the case might be.

In every case at least six determinations were made with each concentration of the salt, and in the absence of agreement between determinations, the series was repeated so that the results as herein reported are in every case the average of four or more closely agreeing determinations. Hence, experimental error has been reduced to as near a minimum as possible in this kind of work.

The solutions of the salts were prepared by weighing gram-molecular quantities of Merck's best grade of the respective salts into 1,000 cc. of sterile distilled water and then quantitatively determining the amount present. In those cases in which the analysis showed the concentration wrong, it was corrected, so that we have a definite knowledge of the quantity of salt added to the soil, as the varying results reported by different investigators can in many cases be interpreted by the unknown variation in salts added.

TABLE 1

Physical and chemical composition of soil

PHYSICAL COMPOSITION		CHEMICAL COMPOSITION	
Soil	Per cent	Constituent	Per cent
Coarse sand (above 1 mm.).......	17.69	Insoluble matter...............	66.69
Fine sand (1 to 0.03 mm.)........	37.39	Potash (K₂O)...................	0.55
Coarse silt (0.03 to 0.01 mm.)....	15.19	Soda (Na₂O)	0.49
Medium silt (0.01 to 0.003 mm.)..	10.36	Lime (CaO)...................	7.41
Fine silt (0.003 to 0.001 mm.)....	10.32	Magnesia (MgO)...............	4.15
Clay (below 0.001 mm.)..........		Ferric oxide (Fe₂O₃)............	2.93
Moisture and loss.............	9.05	Alumina (Al₂O₃)................	3.49
		Phosphorus pentoxide (P₂O₅).....	0.25
		Sulfur trioxide (SO₃)...........	0.07
		Carbon dioxide (CO₂)..........	7.62
		Humus.......................	2.18
		Total nitrogen.................	0.15

The solutions thus prepared were added to the soil and intimately mixed before incubation. Then sodium, potassium, calcium, and magnesium salts were added in quantities sufficient to reduce ammonification or nitrification of the soil between 30 and 50 per cent. The calcium and iron salts were then added in increasing quantities.

Influence of calcium sulfate

Calcium sulfate is a strong soil stimulant and in most cases it greatly increases the crop yield. This is due to a number of factors. First, it may liberate plant-food such as potassium (15) and phosphorus (5); second, it stimulates ammonification (4) to a slight extent and nitrification (6) very markedly; and third, it may under some conditions furnish sulfur to the growing plant. It is often used in the reclaiming of "black alkali" soil, mainly

with the purpose of transforming the black alkali into the white, this latter being more readily leached from the soils. It seems probable that it may at times have an antitoxic effect when applied to alkali soils. Therefore, experiments were conducted in which the following quantities of the respective salts were applied to the soil; sodium sulfate, 2760.2; sodium chloride, 460.0; sodium nitrate, 1840.1; sodium carbonate, 5060.4; magnesium sulfate, 2188.8; magnesium chloride, 972.8; and calcium chloride, 400.64, each stated as parts per million of the cation. This was sufficient to reduce the ammonifying powers of the soil to from 30 to 70 per cent of the original. To this soil was then added calcium sulfate in increasing quantities up to 210 parts per million and the ammonifying powers of the soil determined. The results are given in figure 1.
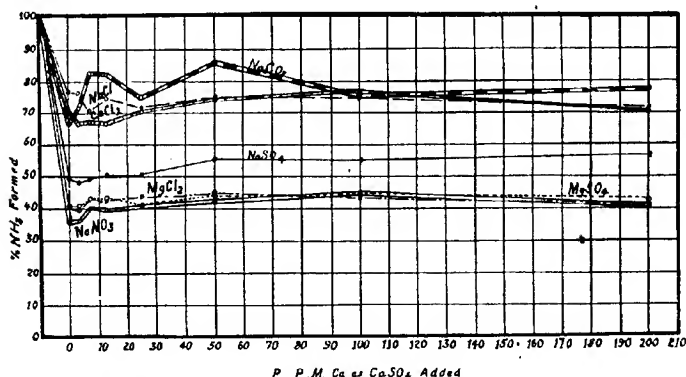
Fig. 1. Diagram Showing the Antagonism of Calcium Sulfate Toward Sodium Chloride, Sodium Carbonate, Sodium Sulfate, Sodium Nitrate, Calcium Chloride, Magnesium Chloride and Magnesium Sulfate, Measured in Terms of Ammonification

A true antagonism exists between calcium sulfate and each salt, except sodium chloride. This is greatest with sodium carbonate at a concentration of 50 parts per million and is largely due to its rendering insoluble the very toxic black alkali. This cannot be the only effect; otherwise, its antitoxic effect should increase until all the sodium carbonate was neutralized. The toxicity is reduced at this point from 33.4 per cent to only 17.1 per cent. The average antagonistic effect is greater toward the univalent cations than it is toward the bivalent cations. Both the anion and the cation apparently exert an antagonism, and in keeping with the findings of Brooks (3) there is a slight antagonistic effect between calcium and magnesium. However, a person would have to conclude from the magnitude of these results that the main practical value of the application of calcium sulfate to soils is in rendering inert the black alkali. Possibly had the salt concentration been lower more marked results would have been obtained.

Nitrification. The same salts were used in the nitrification test as in the ammonification. The concentrations were as follows: sodium sulfate, 1380.0; sodium chloride, 920.0; sodium nitrate, 460.0; sodium carbonate, 1380.0; magnesium sulfate, 729.6; magnesium chloride, 486.4; and calcium chloride, 1602.56, each stated as parts per million of the cation. This was sufficient to reduce the nitrifying powers of the soil from 100 per cent to 27.26 to 71.16 per cent. The results are given in figure 2.

Two salts show no antagonism, sodium sulfate and calcium chloride. The amount noted in the case of sodium chloride is so small that it may be due to experimental error, thus making it rather certain that there is no antagonism between the anions, sulfate and chloride. There is an antagonism between calcium and magnesium in the case of the nitrifiers as well as the ammonifiers.
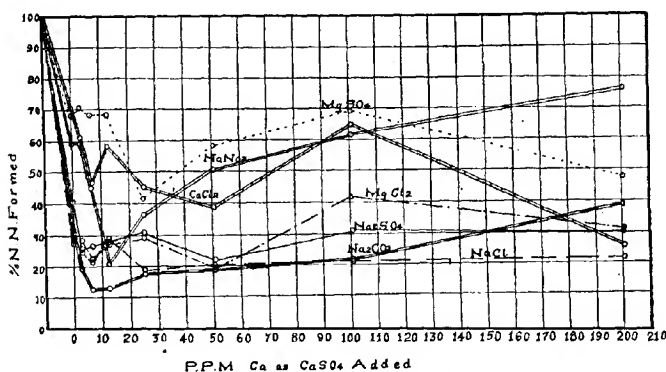
Fig. 2. Diagram Showing the Antagonism of Calcium Sulfate Toward Sodium Chloride, Sodium Carbonate, Sodium Sulfate, Sodium Nitrate, Calcium Chloride, Magnesium Chloride and Magnesium Sulfate, Measured in Terms of Nitrification

It is, however, small in each case. The antitoxic action of the calcium sulfate toward sodium carbonate is not so great as a person might expect, but the results indicate the possibility that if higher concentrations of calcium sulfate had been used the effect would have been more pronounced. The concentration at which the various salts become antitoxic varies markedly. It is evident that the main antagonism is between the cation and not the anion and is slightly more pronounced toward the univalent than the bivalent ions.

Influence of iron salts on ammonification

Iron salts vs. sodium chloride. In this set 460 parts of sodium in the form of sodium chloride was added to the soil. This was sufficient to reduce the ammonium produced to about 75 per cent normal. To these was then added

iron in the form of sulfate, chloride, nitrate and carbonate. The average results from a number of closely agreeing determinations are given in figure 3. The untreated soil has been taken as 100 per cent.
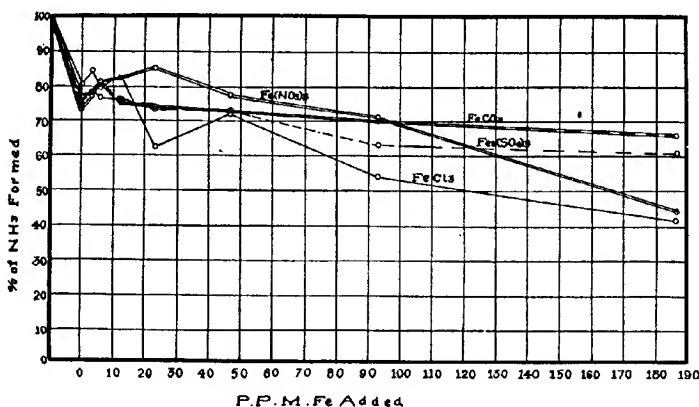
FIG. 3. DIAGRAM SHOWING THE ANTAGONISM OF IRON NITRATE, CARBONATE SULFATE OR CHLORIDE TOWARD SODIUM CHLORIDE, MEASURED IN TERMS OF AMMONIFICATION

Each salt has a slight antagonistic action which is greatest in the case of the nitrate and least in the case of sulfate. The fact that the antagonism does not appear with each salt at the same iron concentration indicates that the anion as well as the cation exerts an action. This, however, is not great for the action is nearly as great in the presence of the common ion chlorine as it is in its absence.
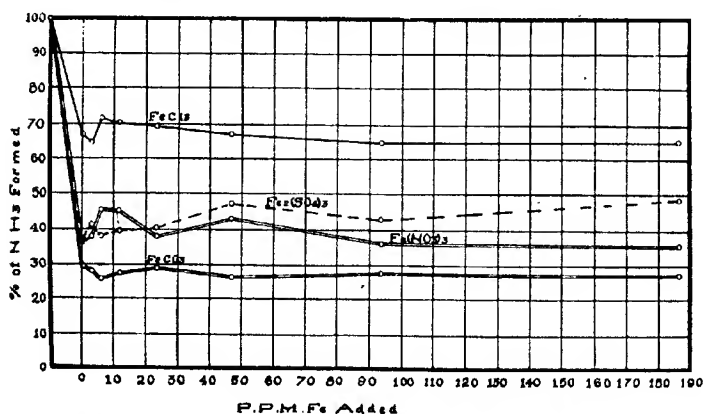
FIG. 4. DIAGRAM SHOWING THE ANTAGONISM OF IRON CHLORIDE SULFATE, NITRATE AND CARBONATE TOWARD SODIUM NITRATE, MEASURED IN TERMS OF AMMONIFICATION

Iron salts vs. sodium nitrate. The same iron salts as used above were tested in combination with sodium nitrate. The results are given in figure 4.

In every case 1840.1 parts per million of sodium in the form of sodium nitrate was added. The iron carbonate had no apparent effect. All others exert an antagonism. This is greatest in the case of the sulfate and least in the case of the chloride. The antagonism is even greater than would appear from these drawings, for when 186 parts of iron is added to the soil in the form of chloride it reduces the ammonifying powers to 72.3 per cent normal; iron sulfate, 84.3 per cent; iron nitrate, 94.8 per cent; and iron carbonate, to 102.1 per cent (4). The increase could not be due to a direct stimulation, for that occurs in much lower iron concentration than the concentration at which antagonism is noted. The iron salts added to a soil even up to 186 parts per million in the presence of sodium nitrate exert no toxic action,
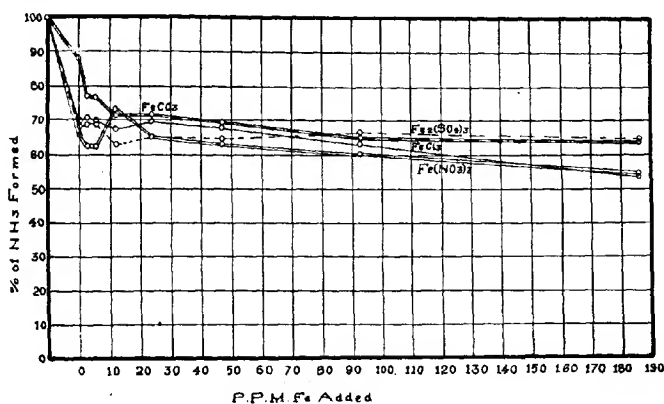
FIG. 5. DIAGRAM SHOWING THE ANTAGONISM OF IRON CARBONATE, SULFATE, CHLORIDE AND NITRATE TOWARD SODIUM SULFATE, MEASURED IN TERMS OF AMMONIFICATION

whereas the same concentration of these salts added to normal soil shows a marked toxic action. The main antagonism is due to the cation, for it is low in the presence of the common anion, carbonate.

Iron salts vs. sodium sulfate. Here the same iron salts were used in the presence of sodium sulfate. The average results for a number of determinations are given in figure 5. The untreated soil is taken as 100 per cent. The quantity of iron added varied from 0 to 186 parts per million. To the soil was added 2760.2 parts per million of sodium in the form of sodium sulfate.

There is no antagonism between the sodium sulfate and iron carbonate and the toxicity of the two salts increases quite rapidly, probably through the formation of sodium carbonate and iron sulfate. Neither the chloride nor the sulfate shows a marked antagonism. The most pronounced antagonism is with the iron nitrate. In this case, however, there is a true antagonism,

for when 11.6 parts per million of iron nitrate was added to this soil (4) its
ammonifying powers were 102.8, whereas when added in the presence of the
sodium sulfate the ammonifying powers were increased 11 per cent. It is
quite evident that in this case the main antagonism is between the anions,
sulfate and nitrate. There is less antagonism between the iron salts and
sodium sulfate than any of the salts so far considered.

Iron salts vs. sodium carbonate. Sodium carbonate was used in combina-
tion with iron carbonate, sulfate, chloride and nitrate. The concentrations
varied from 0 to 186 parts per million of iron. To the soil was added 5060.4
parts per million of sodium in the form of sodium carbonate. The results,
considering the untreated soil as 100 per cent, are given in figure 6.

There is no antagonism between sodium carbonate and iron chloride. The
toxicity of the iron increases just as rapidly in the presence of sodium car-

FIG. 6. DIAGRAM SHOWING THE ANTAGONISM OF IRON SULFATE, CARBONATE, NITRATE AND
CHLORIDE TOWARD SODIUM CARBONATE, MEASURED IN TERMS OF AMMONIFICATION

bonate as in its absence (4). There is a very marked antagonism between
sodium carbonate and iron sulfate. Probably considerable of the benefit
resulting from the sulfate is due to its action upon the physical properties of
the soil, as the soil is rendered light and porous, thus offsetting the extremely
bad puddling effect due to sodium carbonate. Moreover, it is just possible
that the iron sulfate would greatly accelerate the leaching of black alkali
from the soil. The nitrate and carbonate both exhibit marked antagonistic
action toward sodium carbonate.

It is rather evident that there is antagonism between the anion as well as
the cation, for iron carbonate offsets the toxic action of sodium carbonate.
This also may be due to a better aeration in the presence than in the absence
of iron salts. These results give rise to hopes that the iron salts may be used
to advantage in reclaiming black alkali. Pot experiments are now in progress,
the object of which is to answer this question.

Iron salts vs. calcium chloride. The chloride, sulfate, nitrate, and carbonate of iron were added to the soil in quantities such that the iron added with each salt was the same and varied from 0 to 186 parts per million. To the soil was added 400.64 parts per million of calcium in the form of calcium chloride. The results are given in figure 7.

The antagonistic action in each case was extremely small, thus indicating that the antagonism between calcium and iron, as measured in terms of ammonification, is very small. There is, however, a slight antitoxic action between calcium chloride and each other salt, as we note very little decrease in the ammonia produced as the added iron salts increase. Possibly much more pronounced results would have been obtained had smaller quantities of calcium chloride been used, as it is a well-known fact that the concentration of salts may be high enough to prevent antagonism.
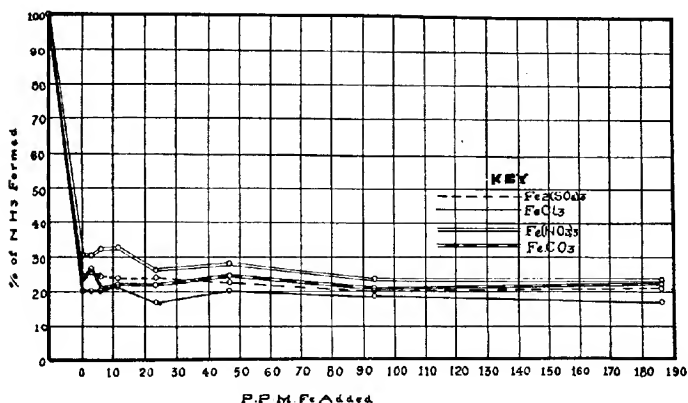
FIG. 7. DIAGRAM SHOWING ANTAGONISM OF IRON SULFATE, CHLORIDE, NITRATE AND CARBONATE TOWARD CALCIUM CHLORIDE, MEASURED IN TERMS OF AMMONIFICATION

Iron salts vs. magnesium chloride. The results obtained when iron salts are used in combination with magnesium chloride are given in figure 8. In combination with iron varying from 0 to 186 parts per million, 972.8 parts per million of magnesium was used.

The only iron salt which increased the ammonification was the nitrate. All the rest ran along about the same, even with increasing quantities of iron. It is, however, quite evident that there is a slight antagonism, for the toxicity of the iron salts does not increase with concentration as is the case when these salts are used singly (4). There is a close resemblance between these results and those obtained with the calcium chloride. There is not so great an antagonism between two bivalent cations as there is between a bivalent and a univalent cation.

FIG. 8. DIAGRAM SHOWING THE ANTAGONISM OF IRON SULFATE, NITRATE, CHLORIDE AND CARBONATE TOWARD MAGNESIUM CHLORIDE, MEASURED IN TERMS OF AMMONIFICATION

Iron salts vs. magnesium sulfate. The various iron salts were used in combination with 2188.8 parts of magnesium as magnesium chloride. The results are reported in figure 9.

FIG. 9. DIAGRAM SHOWING THE ANTAGONISM OF IRON CHLORIDE, CARBONATE, SULFATE AND NITRATE TOWARD MAGNESIUM SULFATE, MEASURED IN TERMS OF AMMONIFICATION

The sulfate does not increase ammonification nor does it become toxic in the presence of magnesium sulfate as it does when used alone (4). The nitrate has only a slight antagonism as compared with the chloride and the carbonate. These latter increase ammonification 31 and 76 per cent, respectively. The antagonism is higher in the case of these compounds than any of the other compounds tested. The results indicate that the chief antagonism is

between the cations, magnesium and iron. Yet the anion must play a part; otherwise, similar results would have been obtained with magnesium chloride. This must be a true antagonism and not due to better aeration, as was suggested as playing a part with sodium carbonate.

Concentration at which iron salts have the greatest action. The concentration at which the various salts have their greatest antagonistic action varies widely with the salt, as is shown graphically in figure 10.

In most cases the antagonism is greatest at very low concentrations; this with sodium chloride, and with the sulfate or chloride of iron is 2.9 parts per million. Assuming an acre-foot of soil to weigh three million pounds, this would require that only 8.7 pounds per acre of iron in the form of the sulfate or chloride to be applied to a soil. Ususally the antagonism is greatest at very low concentrations. For instance, in five combinations it is where the iron concentration is 2.9 parts per million. This would require the addition of 37.6 pounds of iron sulfate, 30.3 pounds of iron chloride, 44.9 pounds of iron nitrate, or 21.9 pounds of iron carbonate. Such applications to one acre of soil, would not make the cost prohibitive, provided later work shows the antagonism to hold with respect to the higher plants. Six combinations show their best antagonism at 5.8 parts per million which would require 75.2, 60.6, 89.8, and 42.8 pounds per acre if the sulfate, chloride, nitrate, or carbonate, respectively, are applied to the soil. However, to get the greatest antagonism between sodium carbonate and iron sulfate it would require that there be applied to the soil 603 pounds of iron sulfate. This quantity markedly changes the physical properties of the soil and very materially modifies its ammonifying and nitrifying powers. It is slightly toxic to the ammonifiers when applied to a normal soil, but in the presence of sodium carbonate increases ammonification 24 per cent. It is quite likely that it would have a similar effect upon some higher plants. Although the antagonism is mainly between cations, it is evident from these results that the anion plays a part.

Extent of antagonism between various salts. The extent of antagonism existing between various salts is given in figure 11. In each case the ammonifying power of the soil when treated with the salts has been considered as 100 per cent.

Six combinations—magnesium chloride vs. iron sulfate, magnesium sulfate vs. iron sulfate, sodium carbonate vs. iron chloride, magnesium chloride vs. iron chloride, sodium nitrate vs. iron carbonate, and sodium sulfate vs. iron carbonate—depress the ammonifying powers of the soil even in the lowest concentrations tested. In the case of most of the combinations the antagonism is slight, but in the case of the more injurious alkalis it is considerable, amounting in the case of sodium carbonate vs. iron sulfate to 24 per cent. This indicates that it may, under appropriate conditions, have an economic value.
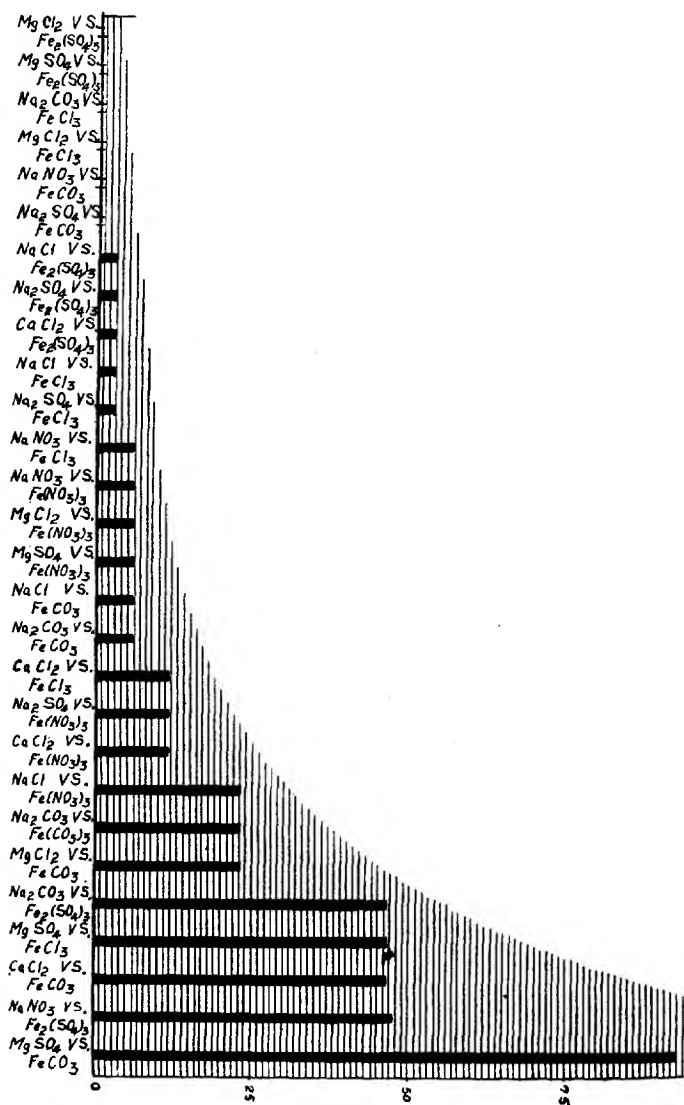
FIG. 10. DIAGRAM SHOWING THE CONCENTRATION OF IRON SALT WHICH EXERTS THE GREATEST ANTAGONISM TOWARD A SPECIFIC SALT, MEASURED IN TERMS OF AMMONIFICATION
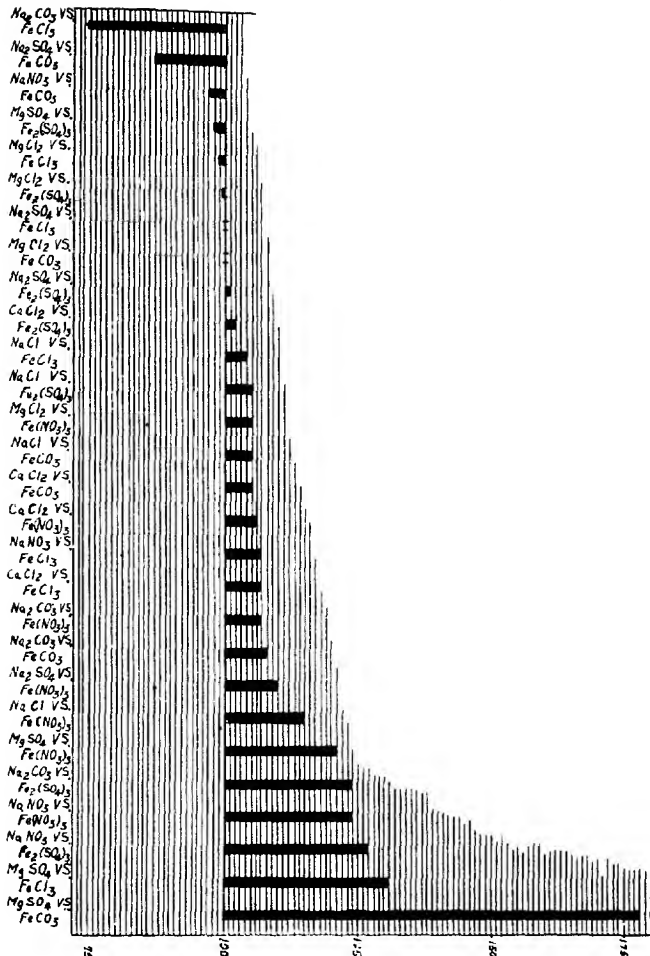
FIG. 11. DIAGRAM SHOWING THE EXTENT OF ANTAGONISM BETWEEN VARIOUS SALTS, MEASURED IN TERMS OF AMMONIFICATION

Nitrification

Iron chloride and iron carbonate stimulate nitrification. Iron sulfate has but little influence, while iron nitrate is a strong poison when used on normal soils (5). But the results obtained on ammonification indicate that the results may be quite different when used in combination with various soil

alkalis. Therefore, determinations were made in which combination of the various iron salts with sodium chloride, sodium sulfate, sodium nitrate, sodium carbonate, magnesium chloride, magnesium sulfate, calcium chloride, or calcium sulfate were used. The general method was that used in ammonification, except incubation lasted 21 days and from four to ten determinations were made. The results as reported consider the untreated soil as producing 100 per cent and are the average of the determinations made.

Iron salts vs. sodium chloride. In combination with iron sulfate, iron nitrate and iron carbonate, 920 parts of sodium in the form of sodium chloride was used. The iron added varied from 0 to 186 parts per million. The results are given in figure 12.

Iron carbonate exerts no antagonism toward sodium chloride as measured by nitrification. The slight gain noted at the higher concentrations is prob-
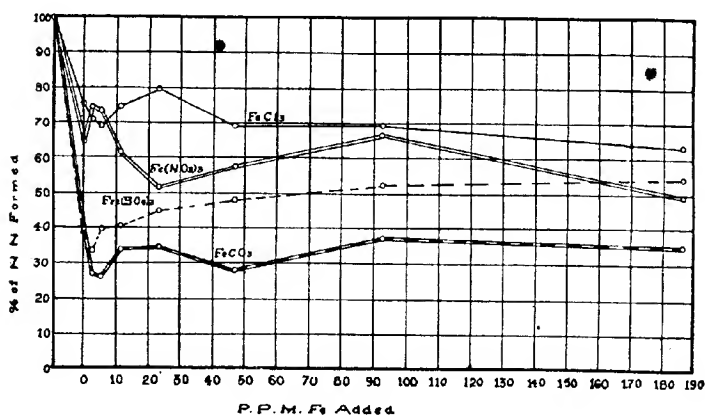
FIG. 12. DIAGRAM SHOWING THE ANTAGONISM OF IRON CHLORIDE, NITRATE, SULFATE AND CARBONATE TOWARD SODIUM CHLORIDE, MEASURED IN TERMS OF NITRIFICATION

ably due to stimulation exerted by the compound, as this compound does stimulate in concentrations from 2.9 up to 1116.9 parts per million. Each of the other salts possesses true antagonistic action, which is greatest with iron sulfate and least with iron nitrate. The variations in concentration at which the iron salts become active make it certain that the anion as well as the cation plays a part. There is also the possibility that a pure physical effect upon the soil may be the main contributing factor. This is borne out by the fact that the iron sulfate is the greatest antagonist but is not so great a stimulant. Although it does not stimulate, yet its antagonistic action increases with concentration. Ferric nitrate, when used alone, is very toxic, yet when used in combination with sodium chloride is not toxic. The chloride and the carbonate are marked soil stimulants as measured in terms of nitrification, yet the carbonate is a better antidote for sodium chloride.

Iron salts vs. sodium nitrate. The results obtained for the iron salts in combination with sodium nitrate are given in figure 13. Four hundred and sixty parts per million of sodium in the form of sodium nitrate was used.

In this set, as in the previous one, iron sulfate possesses the greatest antagonistic action. The nitrate is without effect, whereas the chloride and carbonate neutralized to a slight extent the action of the sodium nitrate. The toxicity of the iron salt increases with concentration even in the presence of sodium nitrate, but not as rapidly as in its absence. The results, together with the appearance of the soil, would lead one to believe that the physical and chemical changes produced in the soil are responsible to a great extent for the neutralizing of the action of the nitrates. But this fact will not materially affect its use in the reclaiming of alkali soils if later tests on soil and
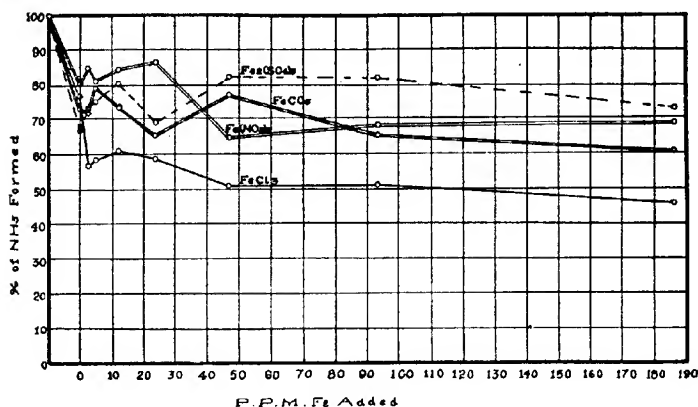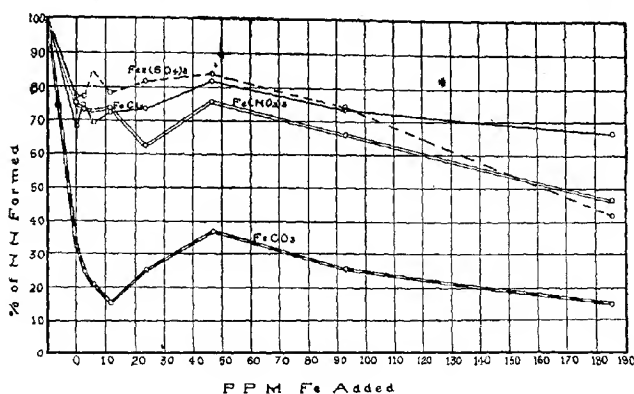

FIG. 13. DIAGRAM SHOWING THE ANTAGONISM OF IRON SULFATE, CHLORIDE, NITRATE AND CARBONATE TOWARD SODIUM NITRATE, MEASURED IN TERMS OF NITRIFICATION

plants show that it increases crop growth or makes the alkali salts more readily leached from the soil.

Iron salts vs. sodium sulfate. The same iron salts were used in combination with sodium sulfate. The results are shown graphically in figure 14. Thirteen hundred and eighty parts per million of sodium in the form of sodium nitrate was used.

The sulfate is without effect, whereas the chloride, nitrate and carbonate all neutralize the toxicity. The antitoxic action of the nitrate and the carbonate is very pronounced. The quantity of iron in the different forms necessary to neutralize varies and is greatest with iron carbonate and least with iron sulfate. Both the anion and cation play a part in the action.

Iron salts vs. sodium carbonate. Such large quantities of sodium carbonate were used in combination with iron chloride and nitrate that little antitoxic

action could be expected, yet the chloride at least possesses a slight antagonistic action, as may be seen from the results given in figure 15.

However, none of the members of this series would lead one to expect great advantages from the use of iron salts on black alkali. Possibly in combina-
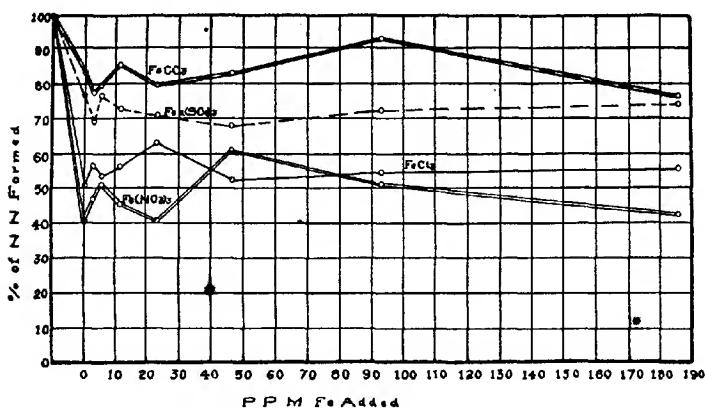
FIG. 14. DIAGRAM SHOWING THE ANTAGONISM OF IRON CARBONATE, SULFATE, CHLORIDE AND NITRATE TOWARD SODIUM SULFATE, MEASURED IN TERMS OF NITRIFICATION
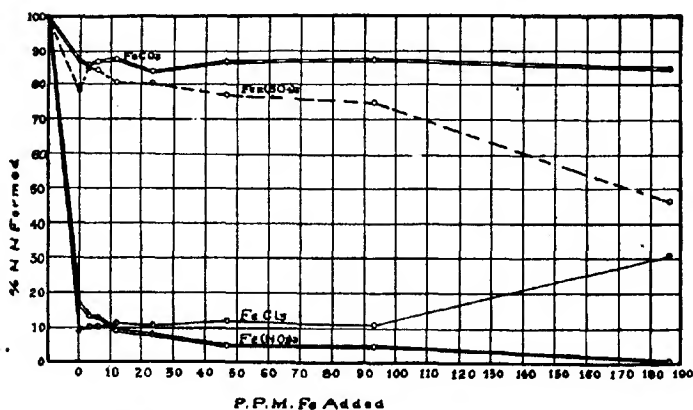
FIG. 15. DIAGRAM SHOWING THE ANTAGONISM OF IRON CARBONATE, SULFATE, CHLORIDE AND NITRATE TOWARD SODIUM CARBONATE, MEASURED IN TERMS OF NITRIFICATION

tion with smaller quantities of sodium carbonate better results would be obtained. But viewing the results with the various sodium salts one must conclude that iron possesses antagonistic action toward sodium, measured both in terms of ammonification and nitrification.

Iron salts vs. magnesium chloride. The various iron salts were used in combination with 486.4 parts of magnesium in the form of magnesium chloride. The results are given in figure 16.

Each of the iron salts acts as a strong antidote to magnesium chloride. The concentration at which they act varies with each specific salt, but in the case of all except the carbonate it is very pronounced. Probably if larger quantities of the carbonate had been used it would have been more pronounced in that case. The concentration at which the iron acts best varies with the negative ion combined with the calcium. This may indicate an antagonism exerted by the anion or a difference in solubility.
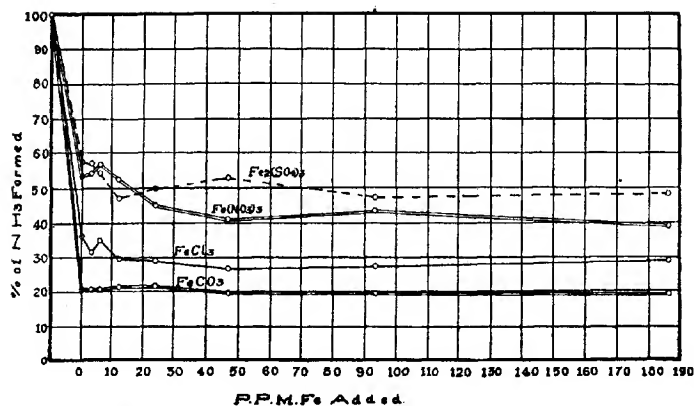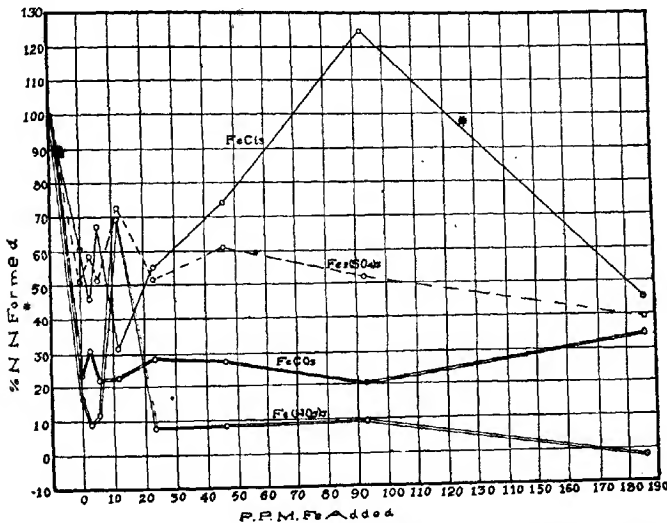

Fig. 16. Diagram Showing the Antagonism of Iron Sulfate, Carbonate, Nitrate and Chloride Toward Magnesium Chloride, Measured in Terms of Nitrification

If experiments with higher plants bear out these results it is quite possible that iron salts may be used effectively and economically in the reclaiming of alkali soils rich in magnesium salts.

Magnesium sulfate vs. iron salts. Practically the same results were obtained with the sulfate as were obtained with the chloride, as may be seen from figure 17.

The carbonate has only a slight effect. All the other iron compounds show marked antagonism to magnesium sulfate, and in each case the concentration is not far different. There is, however, a difference in degree, for iron sulfate is the best antidote for magnesium sulfate; iron chloride is the best antagonist toward magnesium chloride. This bears out the idea suggested in the last

section that plant work may show it to be effective in combating some forms of alkali under a limited known condition.' If effective, it is evident from the low concentration necessary to produce effects that it could be used econom-
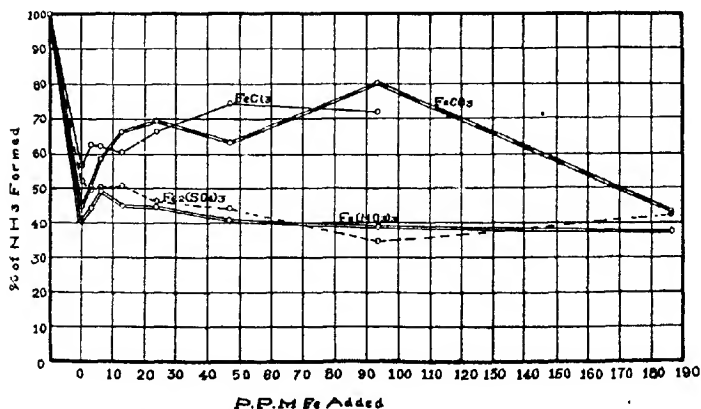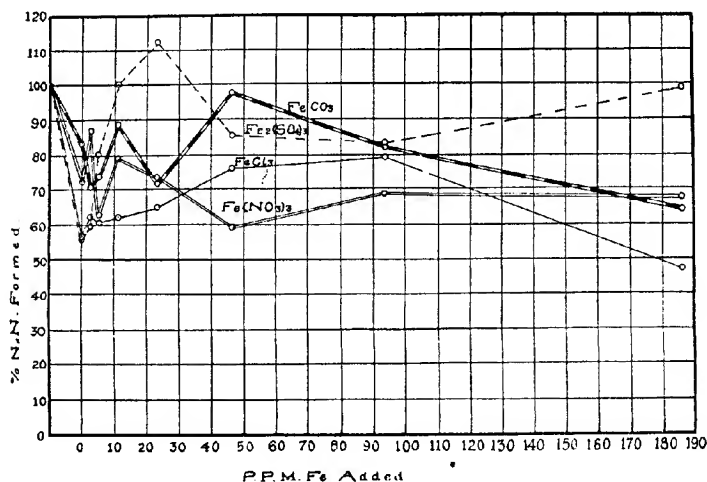

Fig. 17. Diagram Showing the Antagonism of Iron Carbonate, Sulfate, Chloride, and Nitrate Toward Magnesium Sulfate, Measured in Terms of Nitrification
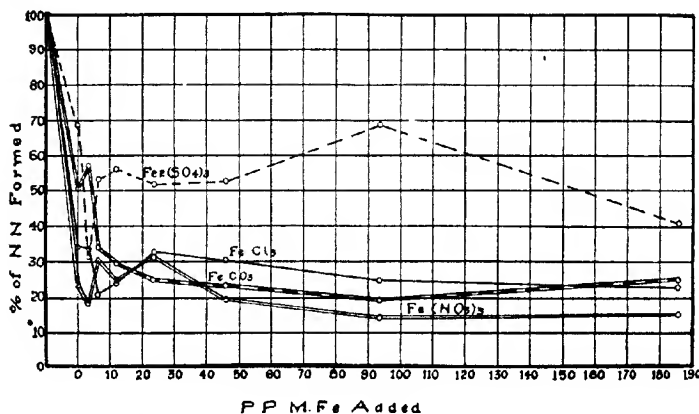
Fig. 18. Diagram Showing the Antagonism of Iron Sulfate, Chloride, Carbonate and Nitrate Toward Calcium Chloride, Measured in Terms of Nitrification

ically. The effect upon the physical condition of the soil was in every case visible and in every case had greatly increased its porosity. This of itself may have played a large part in the change. This, however, cannot be the only factor, for the effect is not directly proportional to the increased aeration.

Iron salts vs. calcium chloride. The results obtained with iron salts in combination with 1602 parts of calcium as calcium chloride are given in figure 18.

Although in none of the concentrations tested did iron sulfate or chloride increase the nitrification over that occurring in the calcium chloride soil, yet it is evident that there must be a slight antagonism since the toxicity of the two salts in combination is not equivalent to that of the two used singly. Iron carbonate, and especially iron nitrate, exert a notable antagonism toward calcium. The results, however, are not as promising with regard to calcium as they are with magnesium.

Concentration at which iron salts have the greatest action. The concentration at which the various iron salts exert their greatest influence varies widely with the iron salt and the alkali, as may be seen from figure 19.

Although in the majority of cases comparatively small quantities of iron are most active as measured in terms of nitrification, yet it has a higher average than is the case with ammonification. This is interesting, for the iron salts are uniformly more toxic to nitrifying (5) organisms than they are to ammonifying organisms (4) when used in the absence of soil alkalis. Only 2.9 parts per million of iron as the sulfate has the greatest influence in combination with the carbonate, whereas 11.6 and 186 parts per million of the carbonate and chloride, respectively, are required. The average quantity of iron in the different forms for greatest antagonism is as follows: iron nitrate, 19.2; iron sulfate, 45.9; iron carbonate, 50.4; and iron chloride, 77.5 parts per million. Should later work on plants show them to have as great a neutralizing influence when measured by plant growth as measured by nitrification, it opens up the possibility of their use in the reclaiming of some alkali soils. The quantities necessary would not be prohibitive in price and they would exert a profound influence upon the physical, chemical, and biological properties of the soil, all of which in so far as our present information goes would be highly beneficial.

The variation in concentration with the different iron salts makes it evident that the anion as well as the cation takes a part in antagonism.

Extent of antagonism between various salts. The extent of antagonism as measured by nitrification between iron salts and soil alkalis is considerable in most cases, as may be seen from figure 20.

Four combinations—sodium carbonate vs. iron nitrate, sodium chloride vs. iron carbonate, calcium chloride vs. iron chloride, and sodium nitrate vs. iron nitrate—depressed nitrification even in the lowest concentrations tested. Sodium sulfate vs. iron sulfate and calcium chloride vs. iron sulfate were without effect, whereas all the remaining combinations stimulated nitrification. In some cases this was high, in a few instances going several hundred times as high in the case of the iron-alkali-treated soil as in the presence of the alkali alone. In those cases where it was highest excessive quantities of the alkali had been added and nitrification was reduced to a great extent; hence, when

expressed in terms of per cent, it appears more pronounced than in the major-
ity of cases.   The order of efficiency of the iron salts as antidotes to alkali
salts is iron chloride, iron nitrate, iron sulfate, and iron carbonate.   The
greatest influence is exerted against magnesium chloride and the least toward
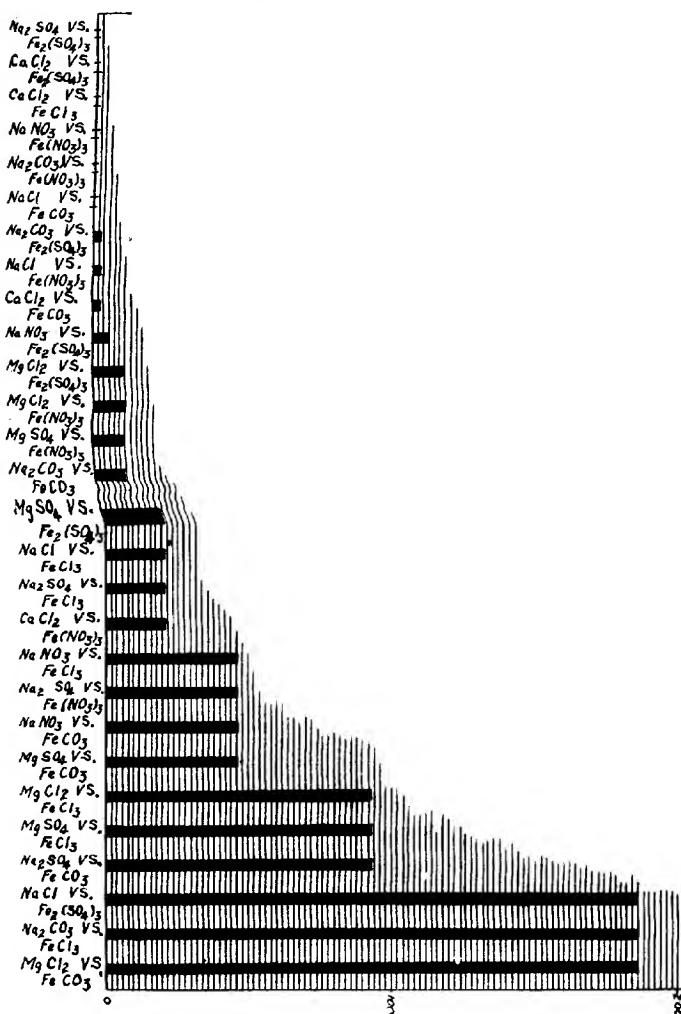
FIG. 19. DIAGRAM SHOWING THE CONCENTRATION AT WHICH VARIOUS IRON SALTS HAVE
THE GREATEST ANTAGONISM TOWARD SPECIFIC SALTS, MEASURED IN TERMS
OF NITRIFICATION

sodium carbonate. With the exception of iron chloride, the influence exerted against the black alkali is not great. However, all average high enough to suggest that they may have some economic significance for the reclaiming of alkali soils.
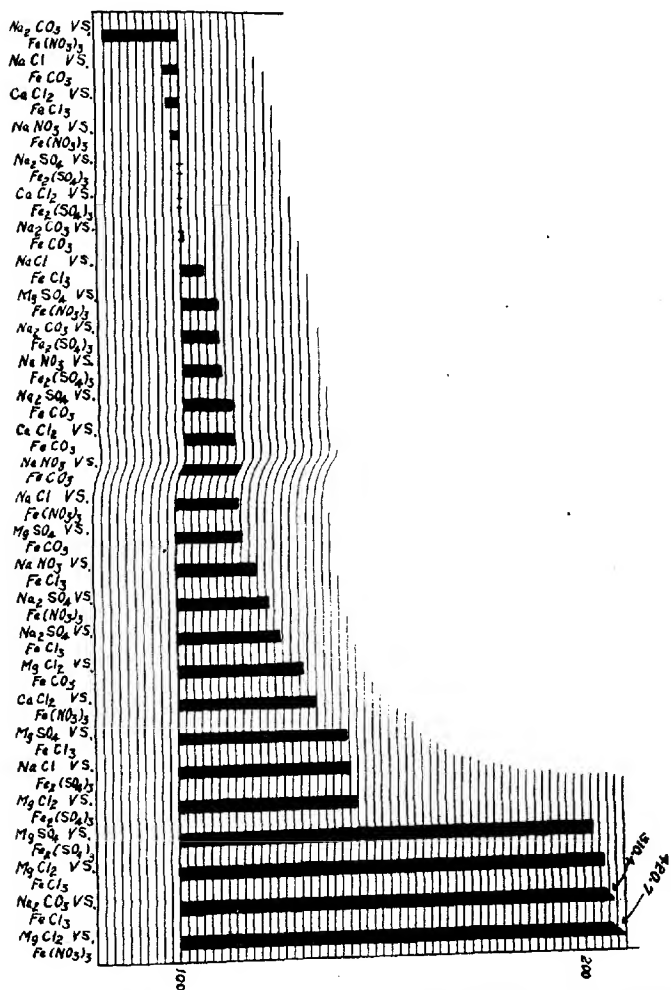
FIG. 20. DIAGRAM SHOWING THE EXTENT OF ANTAGONISM EXERTED BY VARIOUS IRON COMPOUNDS TOWARD OTHER SALTS, MEASURED IN TERMS OF NITRIFICATION

SUMMARY

A true antagonism exists between calcium sulfate and sodium carbonate, sodium nitrate, sodium sulfate, calcium chloride, magnesium chloride, and magnesium sulfate, as measured in terms of ammonification. This is greatest with sodium carbonate and does not occur in the case of sodium chloride. The beneficial effect of calcium sulfate in the presence of sodium carbonate is due to the chemical changing of the sodium carbonate into sodium sulfate and calcium carbonate together with a direct antagonistic action. These results are contrary to those of Lipman (11) and in keeping with those of Brooks (2) in showing an antagonism between calcium and magnesium. This antagonism between calcium and magnesium, although small, also occurs as measured by nitrification.

A similar antagonism exists between these salts, with the exception of sodium sulfate and calcium chloride and calcium sulfate, as measured in terms of nitrification.

These results bear out the findings of Lipman (15) that the anions as well as the cations take a part in antagonism in the case of both ammonifying and nitrifying bacteria.

Iron salts applied to a soil probably change the physical, chemical, and biological nature of that soil. When applied to an alkali soil they usually improve its physical nature, and in this manner offset in a measure the injurious actions of some alkali salts on soils. In addition, some iron salts exert a true antitoxic action toward some alkali salts.

The antagonism is usually greater between monovalent and bivalent ions than it is between two bivalent ions.

As measured in terms of ammonification, a true antagonism was found to exist between sodium sulfate vs. iron sulfate, calcium chloride vs. iron sulfate, sodium chloride vs. iron chloride, sodium chloride vs. iron sulfate, magnesium chloride vs. iron nitrate, sodium chloride vs. iron carbonate, calcium chloride vs. iron carbonate, calcium chloride vs. iron nitrate, sodium nitrate vs. iron chloride, calcium chloride vs. iron chloride, sodium carbonate vs. iron nitrate, sodium carbonate vs. iron carbonate, sodium sulfate vs. iron nitrate, sodium chloride vs. iron nitrate, magnesium sulfate vs. iron nitrate, sodium carbonate vs. iron sulfate, sodium nitrate vs. iron nitrate, sodium nitrate vs. iron sulfate, magnesium sulfate vs. iron chloride, and magnesium sulfate vs. iron carbonate. This was small in the case of the first pair and increased in the order named until the last which neutralized 75 per cent of the toxic effect of magnesium sulfate.

No antagonism was found to exist between sodium carbonate vs. iron chloride, sodium sulfate vs. iron carbonate, sodium nitrate vs. iron carbonate, magnesium sulfate vs. iron sulfate, magnesium chloride vs. iron chloride, magnesium chloride vs. iron sulfate, sodium sulfate vs. iron chloride, and magnesium chloride vs. iron carbonate.

As measured in terms of nitrification, a true antagonism was found to exist between sodium carbonate vs. iron carbonate, sodium chloride vs. iron chloride, magnesium sulfate vs. iron nitrate, sodium carbonate vs. iron sulfate, sodium nitrate vs. iron sulfate, sodium sulfate vs. iron carbonate, calcium chloride vs. iron carbonate, sodium nitrate vs. iron carbonate, sodium chloride vs. iron nitrate, magnesium sulfate vs. iron carbonate, sodium nitrate vs. iron chloride, sodium sulfate vs. iron nitrate, sodium sulfate vs. iron chloride, magnesium chloride vs. iron carbonate, calcium chloride vs. iron nitrate, magnesium sulfate vs. iron chloride, sodium chloride vs. iron sulfate, magnesium chloride vs. iron sulfate, magnesium sulfate vs. iron sulfate, magnesium chloride vs. iron chloride, sodium carbonate vs. iron chloride, and magnesium chloride vs. iron nitrate. This was low in the case of the first pair and increased progressively in the order named up to the last named pair in which the iron nitrate increased the nitrification 420.7 per cent over that soil treated with magnesium-chloride alone.

The quantity of iron required for maximum effect varied with the iron compound and the specific alkali. In no case, however, did the quantity exceed 186 parts per million of iron. Although the greatest influence was exerted by the cations, the anions were not without effect.

If later work shows these salts to have as great an antitoxic action toward alkalis, as measured by higher plants, as has been found to be the case with bacteria they may be used to advantage for the reclaiming of some alkali soils.

REFERENCES

(1) ATKINS, W. R. G. 1916 Some Recent Researches in Plant Physiology. Macmillan, New York and London.

(2) BROOKS, MATILDA M. 1920 Comparative studies on respiration, VIII—The respiration of *Bacillus subtilis* in relation to antagonism. *In* Jour. Gen. Physiol., v. 7, p. 5-15.

(3) BROOKS, MATILDA M. 1920 Comparative studies on respiration, X—Toxic and antagonistic effects of magnesium in relation to the respiration of *Bacillus subtilis*. *In* Jour. Gen. Physiol., v. 7, no. 4, p. 331-336.

(4) GREAVES, J. E. 1916 The influence of salts on the bacterial activities of the soil. *In* Soil Sci., v. 2, p. 443-480.

(5) GREAVES, J. E., AND CARTER, E. G. 1919 The action of some common soil amendments. *In* Soil Sci., v. 7, p. 121-160.

(6) GREAVES, J. E., CARTER, E. G., AND GOLDTHORPE, H. C. 1919 Influence of salts on the nitric nitrogen accumulation in the soil. *In* Jour. Agr. Res., v. 4, p. 107-135.

(7) KELLEY, W. P. 1912 The effects of calcium and magnesium carbonates on some biological transformations of nitrogen in soils. *In* Univ. Cal. Pub. Agr. Sci., v. 1, p. 39-49.

(8) LOEW, O. 1892 Über die Physiologische Function der Calcium und Magnesium Salze in Pflanzenorganismus. *In* Flora, Bd. 75, p. 368.

(9) LOEW, O. 1892 Die Bedeutung der Kalk-Magnesia Salze in der Landwirtschaft. *In* Land. Vers. Stat., Bd. 41, p. 467.

(10) LOEB, J. 1906 Dynamics of Living Matter. New York and London.

(11) LIPMAN, C. B. 1909 Toxic and antagonistic effects of salts as related to ammonification by *Bacillus subtilis*. *In* Bot. Gaz., v. 48, p. 105.

(12) LIPMAN, C. B. 1910 On the lack of antagonism between calcium vs. magnesium and also between calcium vs. sodium. *In* Bot. Gaz., v. 49, p. 41–50.

(13) LIPMAN, C. B. 1913 Antagonism between anions as affecting ammonification in soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, p. 382–394.

(14) LIPMAN, C. B., AND BURGESS, P. S. 1914 The protective action against magnesium carbonate and of calcium carbonate for *Azotobacter chroococcum*. *In* Jour. Agr. Sci., v. 6, p. 484–498.

(15) LIPMAN, C. B., AND GERECKE, W. F. 1918 Does CaCO₃ or CaSO₄ treatment affect the solubility of the soil constituents. *In* Univ. Cal. Pub. Agr. Sci., v. 3, p. 271–282.

(16) OSTERHOUT, W. J. V. 1917 Antagonism and permeability. *In* Science, n. s., v. 40, pp. 97–103.

(17) PAULI, W. 1904 Pharmakodynanische Studien, I. Beziehungen der physiologischen Ester- und Salzquirkung. *In* K. Akad. Wiss. Math. Naturw. Cl. Sitzber., Abt. 3, Bd. 113, p. 15–40. *Quoted from* Stiles and Jorgensen (19).

(18) ROBERTSON, T. B. 1910 Über die Verbindungen der Proteine mit anorganischen Substanzen und ihre Bedeutung für die Lebensvorgänge *In* Ergeb. Physiol., Bd. 10, p. 216–361.

(19) SHEARER, C. 1917 On the toxic action of dilute pure sodium chloride solution on the meningococcus. *In* Proc. Roy. Soc. (London), ser. B, v. 89, p. 440–443.

(20) SHEARER, C. 1919 The action of electrolytes on the electrical conductivity of the bacterial cell and their effect on the rate of migration of these cells in an electric field. *In* Proc. Camb. Phil. Soc., v. 19, p. 263–265.

(21) STILES, WALTER, AND JORGENSEN, INGAVAR 1914 Antagonism between ions in the absorption of salts by plants. *In* New Phytol., v. 13. no. 8, p. 253–268.

(22) SZUCS, J. 1912 Experimentelle Beiträge zu einer Theorie der antagonistischen. Ionenwirkungen. *In* Jahrb. Wiss. Bot., Bd. 52, p. 85.

(23) WINSLOW, C.-E. A., AND FALK, I. S. 1918 Studies on salt ·action: I. Effect of calcium and sodium salts upon the viability of the colon bacillus in water. *In* Proc. Soc. Exp. Biol. Med., v. 15, p. 67.

A CAPILLARY TRANSMISSION CONSTANT AND METHODS OF DETERMINING IT EXPERIMENTALLY

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INTRODUCTORY

The first part of this article is a general theoretical discussion of soil-moisture movement. Following the suggestion of Briggs (1, 2) and the example of Buckingham (3) and Slichter (6), an attempt has been made to attack the problem from the mathematical point of view, making use of well known methods and theorems of mechanics and hydrodynamics. For the benefit of those who are not familiar with the technical language of physics, it may be stated that the investigation assumes the existence of a characteristic soil constant heretofore undefined, a knowledge of which, together with readily obtained experimental quantities, will give sufficient data for the calculation of the direction and magnitude of the capillary stream. For example, it is well known that moisture may flow upward to the surface of the ground more readily from a water-table 6 feet below the surface than from a water-table 12 feet below, and we might assume offhand that the flow would be double from the 6-foot water-table. It is apparent, of course, that the kind and condition of the soil will be concerned, but for a given soil of given structure, it would no doubt be granted that the magnitude of the stream is determined by the moisture gradient and the moisture density, together with the *character* of the soil. The investigation is directly concerned primarily with the matter of defining this *character* in mathematical language and with methods of measuring it experimentally.

In physics we have numerous relations which are more briefly and precisely stated mathematically, in many cases a comparatively simple equation expressing what would require pages in any other language. We have, for example, a mathematical theory of the flow of heat, the flow of electricity, the flow of liquids, gases, etc., including such "usuable" equations as Ohm's law for electricity, Fourier's law for heat, Poiseuille's law for liquids; and in order to make definite progress in moisture studies, the assumption has been made that we must have a general solution for the law of capillary flow.

A network of electrical conductors might be arranged connecting two electrodes and a corresponding current might be experimentally measured for each branch of the network, and a variation of any of the several factors involved, viz., kind and dimensions of wires, number and kind of branches,

potential difference at the terminals, etc., would give an endless variety of experimental curves, each purporting to illustrate some new law, when in reality the entire problem may be solved on the basis of well known fundamental equations by a knowledge of certain characteristic constants of the metals concerned, together with the dimensions of such wires and the nature of the circuit. Likewise, we might succeed in encumbering the literature with innumerable experimental facts, each purporting to illustrate a new law of moisture flow or moisture distribution, when in reality they are all special cases of some general law. It is granted, of course, that experimental investigation must furnish the ultimate answer to this and any other inquiry of the kind, but we should not fail to recognize the fact that classical physics includes much that has already been verified of universal application and there should be little hesitancy in putting this information to use even in such complex problems as those encountered in the study of soils.

The latter part of the article deals with experimental data obtained from various laboratory and field experiments completed and in process at the Utah Experiment Station, planned very largely on the basis of a tentative solution of the problem which has already been published (5). It is hoped that the methods and results may be of interest to the general reader who does not care to enter into an analysis of the theoretical discussion. It is suggested that the transmission constant should be regarded as analogous to the electrical conductivity of a metal wire or to the thermal conductivity of a furnace wall. In the first case we may obtain the strength of the electric current by measuring the potential difference across the ends of a conducting wire if we know the resistance. In the case of the flow of heat we may determine the rate of loss of energy across a furnace wall by measuring the temperature difference if we know the thermal conductivity. Similarly, we may determine the flow of moisture through a soil by measuring the moisture difference between two adjacent points if we know the capillary transmission constant.

THEORETICAL

Briggs (1, 2) pointed out many years ago that the underlying physical factors concerned in the capillary movement of moisture were the surface tension and the coefficient of viscosity of the liquid, together with the geometrical configuration of a rather complex three-phase soil mass. Buckingham (3) proposed a theoretical solution of the problem, introducing a potential to be determined as a function of the moisture density by measuring the moisture distribution in a column of soil containing moisture in equilibrium under the opposing forces due to gravity and capillarity. Slichter (6) has treated the problem where the liquid-air surface is not concerned. Cameron (4) has written an equation for the rate of ascension of capillary moisture. Widtsoe (7) has recognized a law of capillary flow, although he has not expressed it adquately, nor are his data sufficient for its empirical determination.

Through the inspiration of his work, however, the author (5) has proposed a tentative general solution of the problem differing somewhat in method of attack from those indicated.

The problem may be summarized about as follows:—A sheet of water over the surface of a column of dry "dusty" soil in a closed vessel does not constitute a system in equilibrium. A mere drop on such a surface is likewise unstable. In either case, however, in order to pass to equilibrium it may be necessary for movement to take place primarily by distillation, owing to the fact that a transition without a change of state may involve a path in which conditions of relative equilibrium may exist. For example, it may be seen from figure 1 that although the potential at the surface c of a dry particle is lower than at any point within the liquid at a or b except at the solid-liquid boundary, the movement from a to b or b to c except by evaporation and recondensation may involve an actual increase in potential energy. Under ordinary circumstances, however, there is no doubt a continuous channel of a meandering character through which the principal movement may take place without distillation. Neglecting the influence of gravity, the gross
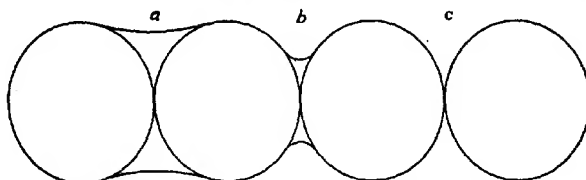
FIG. 1. DIAGRAMMATIC SKETCH OF SOIL-WATER CONFIGURATION IN TWO DIMENSIONS IN AN IDEAL CASE OF UNIFORM SPHERICAL PARTICLES ARRANGED IN LINEAR SEQUENCE

energy of such a system is composed of three types of surface energy, viz., liquid-air, liquid-solid, and solid-air. It should be noted of course that this analysis applies only to a chemically inert system where electrical and chemical energy are in no way concerned. Let these surface energies per unit area be denoted by the symbols, σ_1, σ_2 and σ_3, respectively, and the areas of such surfaces by S_1, S_2 and S_3. The gross surface energy E_σ may then be written.

$$E_\sigma = S_1\sigma_1 + S_2\sigma_2 + S_3\sigma_3 \tag{1}$$

the condition for equilibrium, relative or absolute, being that,

$$\delta E_\sigma = \delta \left(S_1\sigma_1 + S_2\sigma_2 + S_3\sigma_3\right) = 0 \tag{2}$$

If we take into account gravitational energy and denote by h the height measured from a suitable origin, denoting the gravitational energy per unit mass by gh, the effective moisture density or mass per unit aggregate volume by ρ, the volume by v, the gravitational energy integral by E_g, we may write,

$$E_g = g \int_0^{v_1} \rho h dv \tag{3}$$

If, in addition, a part of the air originally occupying the pore space in the soil becomes entrapped, new surfaces may be developed as a result of bubbles forming, etc., and the energy thus involved must be taken into account by proper construction of equation (1).

The gross energy E of the system may therefore be expressed as follows:

$$E = E_\sigma + Eg = S_1\sigma_1 + S_2\sigma_2 + S_3S_3 + g \int_0^{v_1} \rho h dv \qquad (4)$$

The general case may involve each of the several terms of (4). Beneath a shallow mulch, however, in a field soil, it would seem that $S_3 = 0$, and S_2 remains constant, and the two terms, $S_1\sigma_1 + g \int_0^{v_1} \rho h dv$, alone are concerned as variable factors. In the special case of horizontal flow by capillarity the gravitational term becomes fixed and we are concerned only with $S_1\sigma_1$. When the soil is completely saturated $S_1 = 0$ and we have movement by hydrostatic pressure. The latter case has been treated by Slichter (6) and the problem of horizontal capillary flow has been discussed by the author, as above stated.

In a homogeneous insoluble soil with a porosity and arrangement of particles which does not change with the time, there exists a characteristic capillary constant which, with the moisture content and the moisture gradient, determines the magnitude and direction of the capillary current. For a stratified soil, it is apparent that a "compromise" value of such constant must obtain at the boundary, and, for a soil which varies continuously in texture, such transmission function is no longer independent of position, and a calculation of the capillary current at a given point would involve not only a measurement of the moisture content and moisture gradient, but also a measurement of the transmission function for the point in question.

The following equations are quoted from the article[1] (5, p. 314) above cited, where p is written for the pressure due to curvature and v expresses the velocity of the water:

$$\frac{d\rho}{dx} = (K_2/\rho^3)\, dp/dx$$

$$v = (K_3/\eta)\, \frac{dp}{dx}$$

and if it is remembered that the product of the density ρ and the velocity v is flux per unit area, the following relation may be readily derived:

$$K = f\, \rho^3/p$$

where f is written for the magnitude of the capillary stream per unit area and p is written for the moisture density gradient or the differential coefficient $d\rho/dx$, x being a coördinate defining the position of the point considered with respect to any convenient origin, the case considered being the flow in one dimension. The factor K involves implicitly the effective radius of the soil particle, the porosity of the soil, and, as a linear factor, the ratio of the

surface tension and the coefficient of viscosity of the water. It has the dimensions $\frac{M^1L}{T}$. Subject to possible modifications in the original hypotheses this factor may be regarded as a capillary transmission constant.

The direct determination of this constant for a given soil involves the measurement of the moisture density, density gradient, and capillary flow, and we have made preliminary determinations both in the laboratory and in the field by this direct method, although it is laborious.

We have also used a modified method whereby we may obtain the desired information indirectly. A series of curved irrigation sources, convex toward the soil to be irrigated, as illustrated in figure 2, have been provided. These consist of flat galvanized iron pans 1 inch in depth, bounded by two circular and two radial edges as indicated, the water being fed over the top of the "irrigation ditch" by means of an 8-ply linen wick. The water is maintained at a constant level in the ditch and the amount necessary to maintain this level is measured with a burette and recorded with the time. In addition the distance of the water-front as it advances into the soil is recorded. The porosity of the soil is also measured and the density at the source is obtained on the assumption that there is complete saturation. The following calculation will illustrate the method of obtaining the desired information:

If c represents the quantity of water which has moved into the soil from unit area of a vertical section at the source, and if a is the distance the water-front has moved out from the source, and if r is the radius of curvature of the source, then the mean moisture density throughout the wetted area may be expressed as follows:

$$\rho = \frac{\theta c r}{\frac{\theta}{2}(a^2 + 2ar)} = \frac{2cr}{a^2 + 2ar}$$

where θ is the angle of divergence of the circular segment.

The mean density gradient p over this area is

$$p = \frac{\frac{2cr}{a^2 + 2ar} - \rho_0}{\frac{a}{2}} = -\frac{2}{a}\left(\rho_0 - \frac{2cr}{a^2 + 2ar}\right)$$

where ρ_0 represents the moisture density at the irrigation source. The assumption is made that the gradient is constant over the interval a, which is approximately true for small values of a.

A third method involves the measurement of the force moments of the moisture in a series of tubes about an axis through a variable point on the axis of the tube. The distribution of moisture in a soil tube determines the

[1] Attention is called to a factor 2 in equation (2), p. 314, of this article, which should have been omitted; also to the omission of the factor k_2 from equation (7), p. 315.

force-moment about any axis but the converse is not true, and in order to determine the moisture distribution, additional information is needed. For any given distribution the force-moment involves the position of the axis of rotation, as well as the limits of integration in the force-moment integral. If we have a series of soil tubes each of which is bent at right angles, as indicated in figure 3, but the distance of the bend b from the moisture source end increasing regularly from tube to tube, it may be shown by elementary analysis which is omitted for lack of space that the following relation holds:

$$\rho_{(n)} = \frac{M_{(n-1)} - 2M_{(n)} + M_{(n+1)}}{AB^2}$$
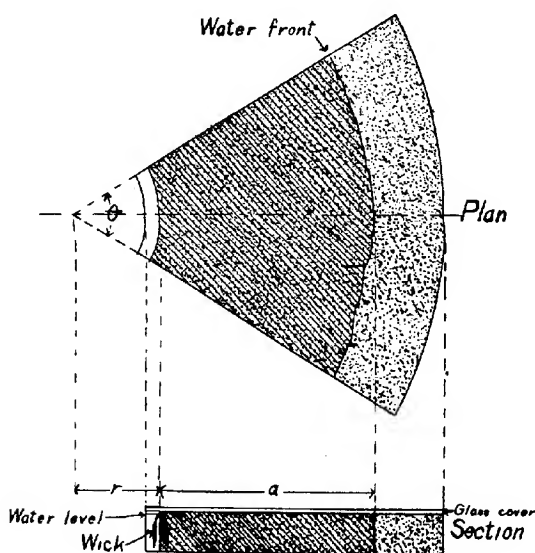
FIG. 2. SKETCH OF SOIL PAN SHOWING RADIAL CHARACTER OF MOISTURE FLOW FROM CIRCULAR SOURCE

where the M's indicate the moments of the moisture beyond the bend about an axis through the point of bending, the subscripts indicating the position of the tube in the series, A the common cross-section of the tubes, B the common difference in the length of branch b from tube to tube of the series, and $\rho_{(n)}$ the average moisture density at the point of bending of the middle one of the series of three tubes involved in the calculation.

A somewhat shorter analysis is given below. If we let x measure the distance of a point from the end at which the moisture enters the tube, a the coördinate of the water front, b the distance to the bend, ρ the moisture

density as previously defined, M the moment, A the cross-sectional area, ρ, x, and b all independent variables, we have,

$$M = A \int_b^a \rho(x - b)dx$$

$$\frac{\partial M}{\partial b} = A \int_b^a \frac{\partial}{\partial b} \left\{ \rho(x - b) \right\} dx = A \int_b^a (-\rho)dx$$

$$\frac{\partial^2 M}{\partial b^2} = A \rho_b$$

$a = b + d$
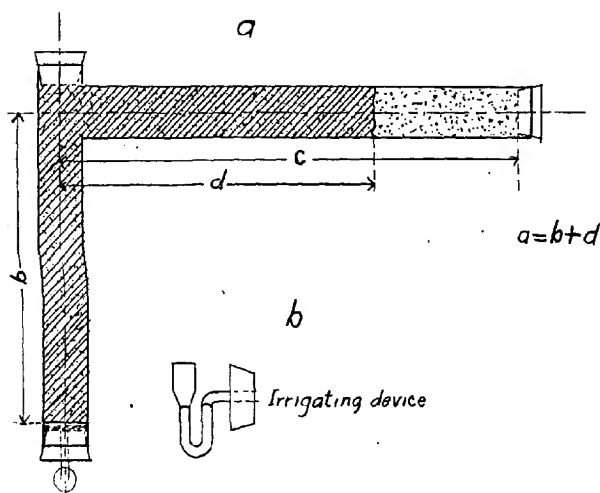
FIG. 3. DIAGRAM SHOWING CONSTRUCTION OF SOIL TUBE

The moment of the moisture in branch c about the axis of branch b is measured for a series of tubes with varying length of the respective branches.

We have had made a series of sectional capillary tubes, each provided with a T, the sections being supplied with a longitudinal window of celluloid. These tubes have been balanced in such a way that the force-moment may be observed from time to time, together with the coördinate of the water front, the respective tubes differing only in the relative lengths of arms b and c (fig. 3) of the tubes. From these data we can determine the relation between M and b as M varies with the time, and then, from equation (1), we may calculate the value of ρ for a series of points along the tubes. A knowledge of ρ as a function of x in which the time enters as a variable parameter is sufficient for an evaluation of the transmission constant, provided a sufficient range of values of ρ and p is obtained.

Before discussing the preliminary results reported below, it may be of interest to point to some well known experimental facts bearing on the general validity of assumptions made in the theoretical solution cited above. Experimental data are available from various sources, exhibiting the form of the function involving the quantity of capillary moisture absorbed and the time, also the coördinate of the water front and the time. These functions appear to be of parabolic form and it will be noted that the following development upon theoretical grounds leads to simple parabolae.

Let us consider any point b in a tube of soil carrying capillary moisture. Let Q represent the moisture which has passed beyond this point at any given time t. This may be expressed as the integral of the density ρ between the limits a and O, where a is the coördinate of the water front measured from the point b taken as the origin, thus,

$$Q = \int_0^a \rho dx \qquad\qquad \text{(A)}$$

If we may assume that $\rho = (Ax + B)^{\frac{3}{2}}$ (see article cited, p. 315, which purports to be true only for a *steady* state, the integral is readily obtained, giving,

$$Q = \frac{2}{5A}\left[(Ax + B)^{\frac{5}{2}}\right]_0^a \qquad\qquad \text{(B)}$$

Expanded, this becomes,

$$Q = \frac{2}{5A}\left[B^{\frac{5}{2}} + \frac{5}{2}B^{\frac{3}{2}}Ax + \frac{\frac{5}{2}\times\frac{3}{2}}{\underline{|2}}B^{\frac{1}{2}}(Ax)^2 + \ldots\right]_0^a \text{(C)}$$

a converging power series in (Ax) which, upon substitution of limits and neglecting terms in $(Ax)^3$ and beyond, becomes,

$$Q = B^{\frac{1}{2}}a\left(B + \frac{3}{4}Aa\right) \qquad\qquad \text{(D)}$$

where
$$A = \frac{\rho_a^{\frac{1}{2}} - \rho_o^{\frac{1}{2}}}{a} \qquad\qquad \text{(a negative quantity)}$$

and
$$B = \rho_o^{\frac{1}{2}}$$

the subscripts serving to define the point concerned. Substituting for A and B their values as thus expressed, we obtain

$$Q = a\frac{(\rho_0 + 3\rho_o^{\frac{1}{2}}\rho_a^{\frac{1}{2}})}{4} = \int_0^{t_1} f dt \qquad\qquad \text{(E)}$$

where f expresses the flow across unit area in unit time. Writing for brevity

$$c_1 = \frac{\rho_0 + 3\rho_0^{\frac{1}{3}}\rho_a^{\frac{2}{3}}}{4}$$

this becomes,

$$Q = c_1 a = \int_0^{t_1} f\,dt \tag{1}$$

If c_1 is independent of a, which is true only when ρ_a does not appreciably change with a, we obtain, upon differentiation,

$$da/dt = f/c_1 \tag{2}$$

From equations (4) and (5), p. 314, of the article cited, we have

$$\rho v = f = \left(K \frac{d\rho}{dx} \right) \Big/ \rho^{\frac{1}{3}} \tag{3}$$

where K is the transmission constant previously referred to. Eliminating f from (2) and (3), we obtain,

$$da/dt = (K/c_1) \frac{d\rho}{dx} \Big/ \rho^{\frac{1}{3}} \tag{4}$$

the density and density gradient being the values for the point b. Differentiating the equation,

$$\rho = (Ax + B)^{\frac{3}{2}}$$

we obtain,

$$\frac{d\rho}{dx} = \frac{3A}{2} (Ax + B)^{\frac{1}{2}} = \frac{3}{2} AB^{\frac{1}{2}} \text{ for } x = 0$$

Substituting again for A and B, we have,

$$\frac{d\rho}{dx} = \frac{3}{2} \frac{(\rho_a^{\frac{1}{3}}\rho_0^{\frac{2}{3}} - \rho_0)}{a}$$

Writing for convenience,

$$c_2 = \frac{3}{2} (\rho_a^{\frac{1}{3}}\rho_0^{\frac{2}{3}} - \rho_0)$$

we obtain,

$$\frac{da}{dt} = \left(\frac{Kc_2}{c_1\rho_0^{\frac{1}{3}}} \right) \left(\frac{1}{a} \right)$$

If we write again for brevity,

$$\phi = \frac{c_2}{c_1\rho_0^{\frac{1}{3}}} = \frac{6(\rho_a^{\frac{1}{3}} - \rho_0^{\frac{1}{3}})}{\rho_0 + 3\rho_0^{\frac{1}{3}}\rho_a^{\frac{2}{3}}}$$

we obtain finally

$$a^2 = 2 K\phi t \tag{5}$$

Or, again, from (1),

$$a = Q/c_1$$

whence,

$$Q^2 = 2K\phi c_1^2 t$$

or

$$Q^2 = 2 K\theta t \tag{6}$$

where

$$\theta = \phi c_1^2 = \frac{3}{8} \rho_0^\frac{1}{3}(3\rho_a^\frac{4}{3} - 2\rho_0^\frac{1}{3}\rho_a^\frac{1}{3} - \rho_0^\frac{4}{3})$$

It is true of course that ϕ and θ may depend upon a, but, as stated, where ρ_a does not appreciably change with a these functions may be regarded as independent of a.

DISCUSSION OF EXPERIMENTAL RESULTS

A considerable amount of experimental data have been obtained from our laboratory work, and in addition some work has been done in the field at the experimental farm at Greenville, and the prinicpal results have been summarized in the following pages.

In figure 4A is plotted a series of curves representing the moisture distribution as it changed with the time in a small rectangular box of soil 6 by 9 cm. in cross-sectional area, one end of which was bent downward and kept permanently in contact with free water maintained at a constant height about 10 cm. below the center of the box. Samples were taken with a small cork borer at frequent intervals and moisture determinations were made by the usual method of weighing and drying. The data represent the mean of observations taken from duplicate boxes. The vertical lines are drawn at distances corresponding to the position of the water front at various intervals of time and the point of intersection of these lines with the distribution curves of corresponding date should determine the moisture per cent at the water front. From the fact that these values appeared to vary irregularly about a mean as the water front advanced, the mean value was assumed to represent a constant moisture per cent at the water front. In reality this would doubtless diminish slowly with the time. In plot 4B is shown a series of straight lines passing through a common point on the moisture per cent axis representing the moisture per cent at the source. Each curve of the series was determined by this common point and the point of intersection of the horizontal line representing the moisture per cent at the water front with a vertical line marking the position of the water front at the time. The area bounded by each curve of the family, the axes of coördinates, and the vertical line, is proportional to the quantity of moisture in the soil at the time represented by the vertical line, and the difference in this area for successive curves divided by the time interval, represents the mean flow into the soil during the interval. The moisture gradient is proportional to the slope of the mean distribution curve over the interval, and the moisture density is proportional to the ordi-
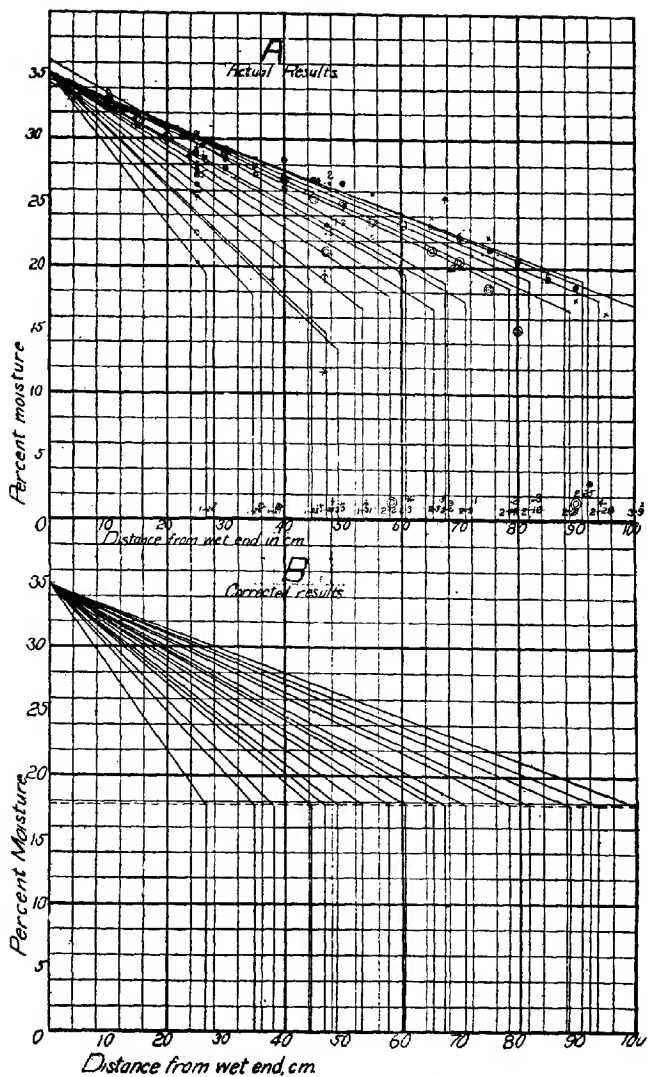
FIG. 4. MOISTURE-DISTRIBUTION CURVES IN LABORATORY TEST

The curves at the top are drawn to illustrate the moisture distribution at successive intervals in a laboratory test of the horizontal capillary flow. Below is shown a series of curves passing through a common point and terminating in a horizontal line representing the average moisture content at the water front. The point of intersection of each line of the series with this horizontal line was determined by a vertical line drawn at a distance corresponding to the position of the water front for a given time. If the moisture distribution were a linear function of the distance these curves would give the history of the moisture in this case.

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nate of the curve. It may be noted, however, that the functions ϕ and θ previously defined were determined by the value of the ordinate of the common point and the constant moisture per cent at the water front. Sufficient data are therefore available for the calculation of K by use of the equation,

$$Q^2 = 2K\theta t$$

and in column 1 of table 1 are recorded a series of values of K thus calculated.

TABLE 1

Values of capillary transmission constant for Greenville soil

Columns 1 and 2 represent values obtained from a very loose unpacked soil from data obtained by the direct method; columns 3, 4, 5 and 6, from the same soil packed dry by systematic shaking, data obtained from the second method; column 7 the direct method from a field plot; columns 8, 9, 10 and 11, from the same soil well packed by wetting and drying, from the second method*

1	2	3	4	5	6	7	8	9	10	11
1.3	2.5	11.9	7.4	7.8	7.6	6.3	1.4	10.7	2.8	2.9
1.6	2.0	13.3	8.7	7.9	8.0	5.9	1.3	8.7	2.6	2.8
1.6	2.0	14.8	9.4	7.9	8.1	10.2	2.1	6.7	2.5	2.6
1.9	1.9	15.8	9.7	7.7	8.5	13.0	3.2	5.8	2.4	2.6
1.8	1.8	17.4	10.5	7.6	8.0		3.7	7.1	2.4	2.7
1.8	1.7	16.6	10.7	7.3	8.3		4.1	9.6	2.6	3.5
1.9	1.5	17.0	11.4	6.8	7.2		4.7	12.4	3.5	4.3
1.9	1.2	14.4	12.1	7.3	8.2			12.3	3.8	5.1
1.9		18.0	15.0	8.0	8.1			14.0	5.7	4.9
1.9		15.4	13.0	9.1	9.0				6.6	4.3
1.9			13.1	7.7	9.3				7.1	4.6
1.8			13.2	9.4	10.3			.	9.4	5.4
1.8			13.7	8.8	11.2					6.5
1.5			14.3	8.8	10.7					
1.7										
1.6										
1.6										
1.4										
1.7	1.8	15.5	11.6	8.0	8.8	8.7	2.9	9.7	4.8	4.1

* It should be noted that columns 3, 4, 5, 6, 8, 9, 10 and 11 involved the measurement of the depth of a column of soil of approximately one inch in depth and the precision of this measurement was not entirely satisfactory. Differences, therefore, from column to column may involve considerable experimental error, whereas the variation in each individual column is not dependent upon this measurement.

In order to convert moisture percentages as plotted to moisture densities in c. g. s. units, an apparent specific gravity of the soil of 1.35 gm. per cubic centimeter was used, the value of θ being -0.118 in c. g. s. units. The values given in the table should be multiplied by $-10.^{-3}$ to convert them into c. g. s. units.

In column 2 of table 1 is shown a series of values ranging from 2.5 to 1.2, calculated from the formula,

$$a^2 = 2K \phi t$$

where a is the distance in centimeters of the water front from the wet end of the soil column, t is the time in seconds measured from the beginning of the experiment, and $\phi = -1.0$ in c. g. s. units.

In figure 5 is shown a series of curves representing the moisture distribution in a field plot, the soil being the same as that used in the laboratory. This plot was kept saturated at the surface by continuous sprinkling, and moisture determinations were made during and after the sprinkling at frequent intervals for several weeks. The data obtained from July 31 to August 11 are shown on the plot. The sprinkling was discontinued at the latter date. The curve at the bottom, which is nearly horizontal, represents the moisture condition when the experiment was begun and the other curves represent the condition on July 31, August 2, August 4, August 7, and August 11, respectively. The area bounded by the moisture per cent axis, the original distribution curve, and any curve of the series is proportional to the quantity of moisture which had moved into the soil since the beginning of the experiment. From the equation

$$K = \frac{f \rho^{\frac{1}{2}}}{p}$$

values of K were obtained from the data of figure 5 ranging from 6.3 to 13.0, as recorded in the seventh column of table 1.

Data obtained from the second method are plotted in figures 6A and 6B. As indicated elsewhere in this article, the gradient and the moisture density may involve the radius of curvature of the irrigation source and a wider range of values was obtained by using several pans differing in this respect. Pan no. 1 had a radius of 2.54 cm., no. 2, 7.62 cm., no. 3, 15.24 cm., no. 4, 30.48 cm., no. 5, 60.96 cm., no. 6. 121.92 cm., and no. 7 had no curvature. Two curves have been plotted for each pan, the one representing the total quantity fed into the soil per unit area of the irrigation source, and the time, the other the distance of the water front from the source and the time. Values obtained from the plot, together with a value for ρ_0 of 0.48 as determined on the assumption that the 48 per cent of pore space was entirely full at the source, were substituted in the equation

$$K = \frac{f \rho_0^{\frac{1}{2}}}{p}$$

and in columns 8, 9, 10 and 11 of table 1 are recorded the values obtained from the data in figure 6A. The soil was well packed in this case by a previous thorough wetting and drying. A slight leak through two of the pans, no. 4 and no. 5, and an accident with no. 3, made the data for these cases uncertain and no attempt was made to use them.

WILLARD GARDNER
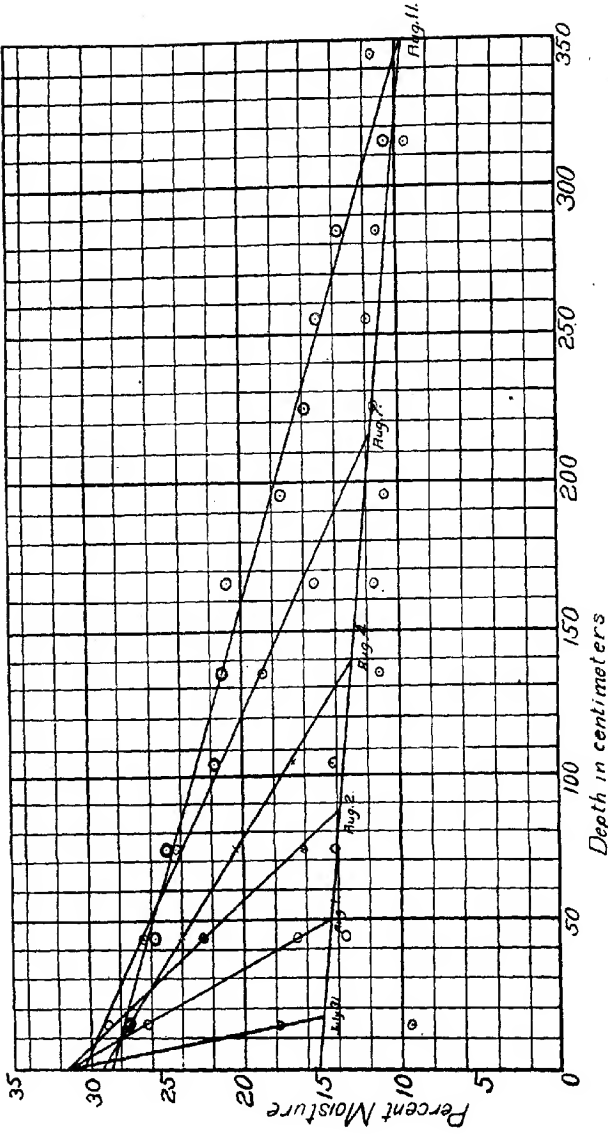
FIG. 5. MOISTURE-DISTRIBUTION CURVES IN FIELD PLOT TEST

The curves in this plot represent the moisture distribution measured at frequent intervals in a field plot where the surface was kept constantly saturated by use of a sprinkler.
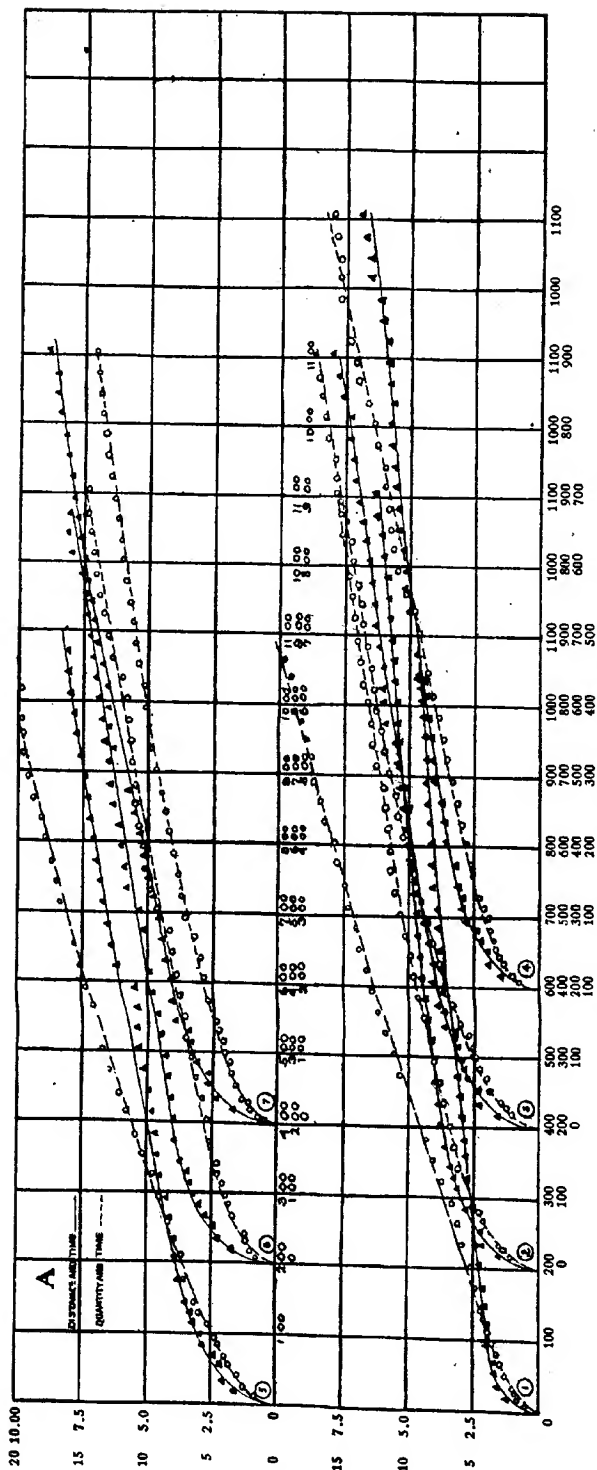
FIG. 6. CURVES ILLUSTRATING THE MOVEMENT OF MOISTURE INTO A RADIAL SECTOR OF SOIL

Two sets of curves are drawn, the one indicating the quantity of moisture which has moved across the unit area of the circular source as a function of the time, the other the distance of the water front from the source as a function of the time.

B

Mean. Distilled Acid Time _ _ _ _ _

Mean Quantity and Time. _____

12.5

10.0

7.5

5.0

2.5

0

118
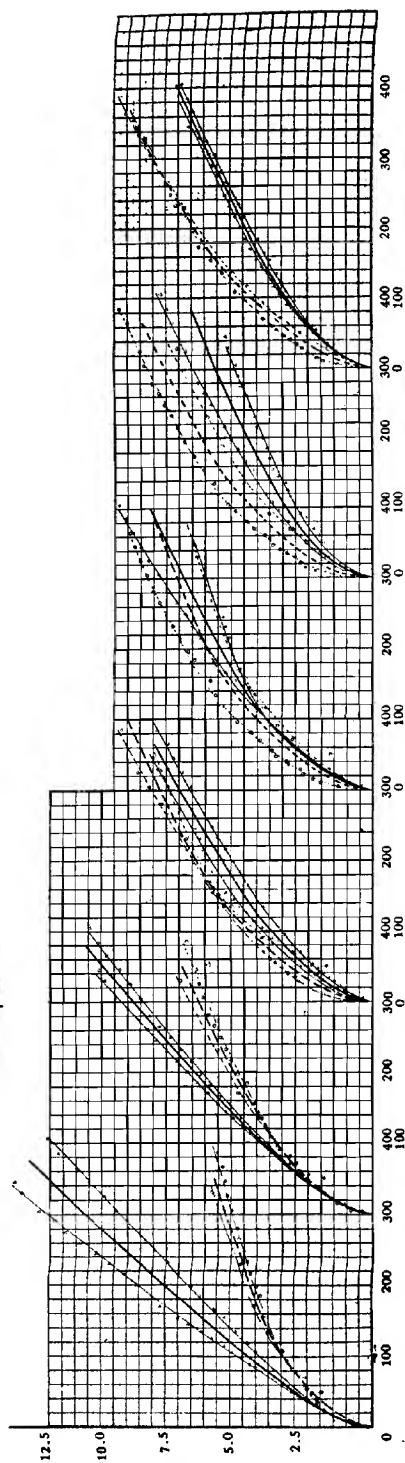
In figure 6B similar results are shown for duplicate trials with the first six pans of the series. The soil in this case was packed without wetting by systematic shaking. The heavy lines represent the mean of two observations, the separate observations being shown on the plot with lighter curves. In columns 3, 4, 5 and 6 of table 1 are recorded values of K calculated from
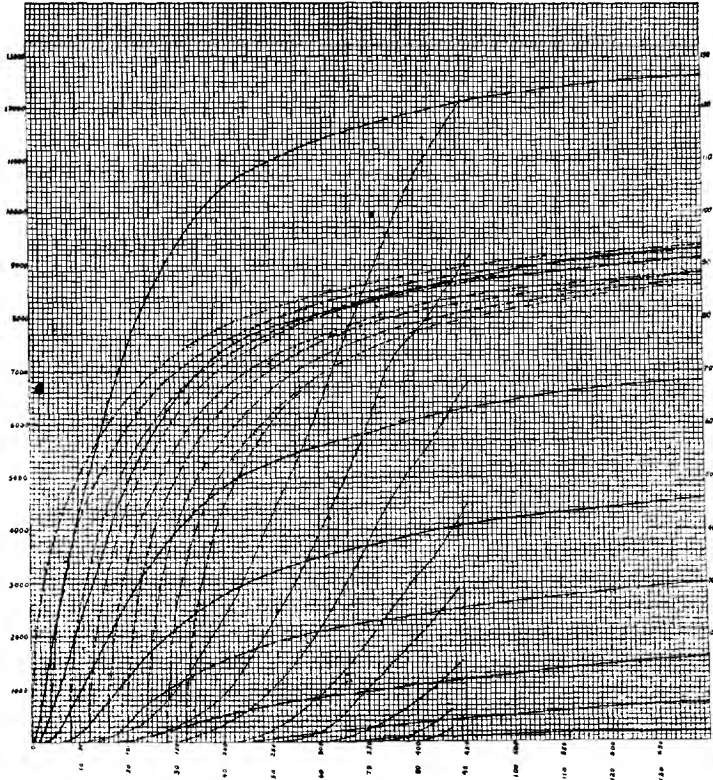
FIG. 7. A PLOT OF DATA OBTAINED FROM A SERIES OF CAPILLARY TUBES

The solid lines represent moment-time data; the broken line, distance of water front from source and time; the light solid lines are the moments plotted against the water front distance.

the data of this plot from pans 1, 2, 5 and 6. Trouble was experienced again with pans 3 and 4 and calculations were not made for these.

In figure 7 are plotted three types of curves representing the data obtained from the third method. A series of nine brass capillary tubes of approximately 5 cm. internal diameter was used. The first was bent at a point 10 cm. from

the end, the second 20 cm., the third 30 cm., and so on. These were balanced in such a way that the moment about an axis through the "source" branch of the tube could be measured with a precision of 1 to 5 centimeter-grams. The series of heavy solid lines are moment-time curves, the broken lines representing the distance of the water front from the wet end and the time; and the light solid lines are the moments plotted against the water front distance. The ordinate represents centimeter-grams with the moment curves and centimeters with the others. The abscissa represents time in hours in two cases and distance in centimeters in the other. The origin is the same for all except the distance-time curves, each of which was shifted horizontally 20 hours from the next preceding curve. These latter curves should of course be strictly parallel except for unavoidable differences in the various tubes. The data for the curves representing the moments plotted against the water-front distances were taken from the other curves rather than from the original tabulated results since these curves eliminate the small oscillations and experimental errors. It will be noted that the ordinates of the points of intersection of a vertical line with the various moment-distance curves represent the simultaneous moments of the moisture in the various tubes. As previously shown, the mean moisture density over the interval between alternate bends may be obtained by adding the simultaneous moments for such alternate tubes and subtracting from the sum twice the moment of the intermediate tube and dividing the result by the product of the cross-sectional area of the tubes (17.7 sq. cm.²) and the square of the bend increment (i.e., the common difference in length from irrigation source to bend between successive tubes—10 cm. in this case).

In figure 8A is plotted a series of curves representing the history of the moisture in the several tubes, the data for which were obtained from figure 7 as above explained. The points at the end of each of the several curves enclosed with large circles mark the intersections of vertical lines representing the position of the water front and straight lines passing through a common point representing the moisture density at the wet end of the tube. These straight lines were drawn to best represent the somewhat irregular moisture densities along the tube. The extrapolated points are taken as indicating approximately the moisture content at the water front. The heavy solid line is drawn to indicate the condition at the water front, and in figure 8B this same line has been drawn and the points of intersection of this line with the vertical lines marking the position of the water front at various times, together with the moisture content at the wet end of the tubes, have been taken to determine a series of approximately straight lines purporting to represent more nearly the actual history of the moisture in the tubes. From this plot the constant ϕ has been determined and from this the transmission constant has been calculated, averaging -4.7×10^{-3} c. g. s. units.

The curves in 8A have been drawn in zig-zag fashion in order more clearly to indicate the actual points determined from the data of figure 7. The wide

irregular variation from point to point may be partly explained by assuming
that considerable amounts of air were entrapped at the wet end of the tube
at the beginning of the experiment, the effect of which would be made
manifest in actual fluctuations in the moisture content. In order to
eliminate as much as possible accidental errors due to irregularities in indi-
vidual tubes, results were calculated for a series of points by a particular
combination of tubes and the mean of successive values was then taken to
indicate the moisture content at intermediate points, and a consideration of
the precision of the experiment indicates that these marked differences cannot
be entirely accounted for by experimental error.

Without attempting to give a detailed account of the measure of precision
of the experiment, it may be noted that by allowing an error of 0.01 cm. in
the length of the lever arms of the levers which were used to balance the
tubes, 0.01 gm. in the weights, 0.01 mm. in the diameter of the tubes, and
0.01 cm. in the length of the bend increment of the tubes as previously
referred to, the probable error of an individual determination of the density
should not exceed 0.1 gm. per cubic centimeter. For actual variations with
the time for determinations involving any given combination of tubes the
only experimental error involved is the error in the weights used to balance
the tubes. The formulae used were those given by Mellor in his "Higher
Mathematics for Students of Chemistry and Physics" and the calculations
were made on the assumption that each individual determination of the
density involved the sum of three independent terms. An increase in the
actual number of tubes used in the series would materially increase the relia-
bility of results obtained.

The tubes were irrigated with measured quantities of water, all receiving
the same amount. Small glass tubes, bent as illustrated in figure 3B, were
used for irrigating the soil, the aim being to maintain the moisture in the bend
at such a constant height as to avoid hydrostatic pressure and at the same
time provide available free water for the end of the soil column. The rate of
application of water proved somewhat arbitrary, however, since it was diffi-
cult to maintain a definite height in any of the various tubes. It can not
be said, therefore, that the curves of figure 7 representing the position of the
water front at different times are exactly as they should have been had the
wet end of the tubes been maintained constant by some other device. From
the mean of these curves, however, calculations of the constant were made by
means of the formula,

$$a^2 = 2 K \phi t$$

the mean value of the constant ϕ (−1.4) being determined as previously
shown from the data of figure 8B. In table 2 is given a series of values of
K thus obtained. It will be noted that there is a decrease from top to bottom
of the various columns. This may be partly accounted for from the fact that
this formula was derived by neglecting terms in a converging series involving
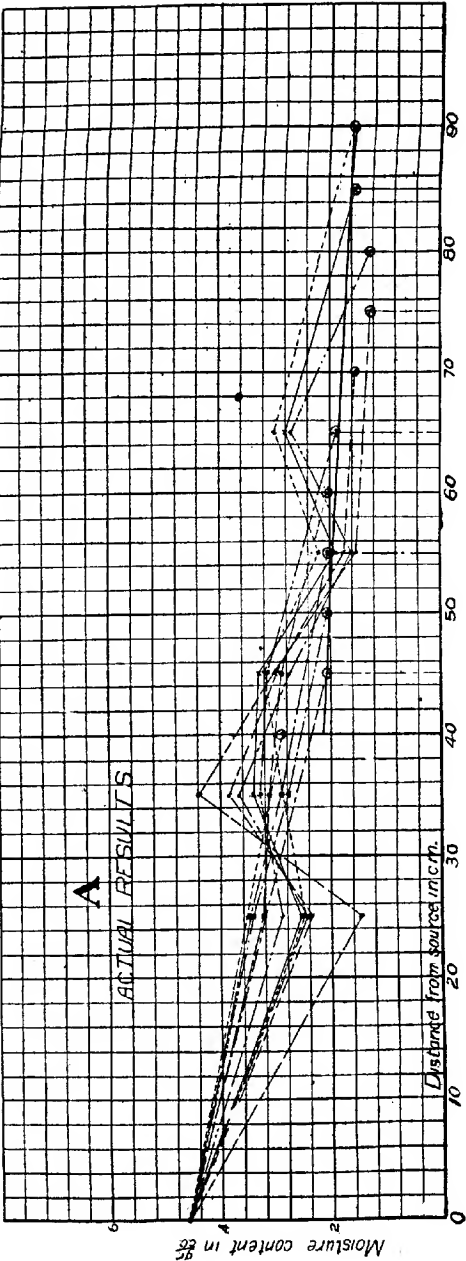
Fig. 8A
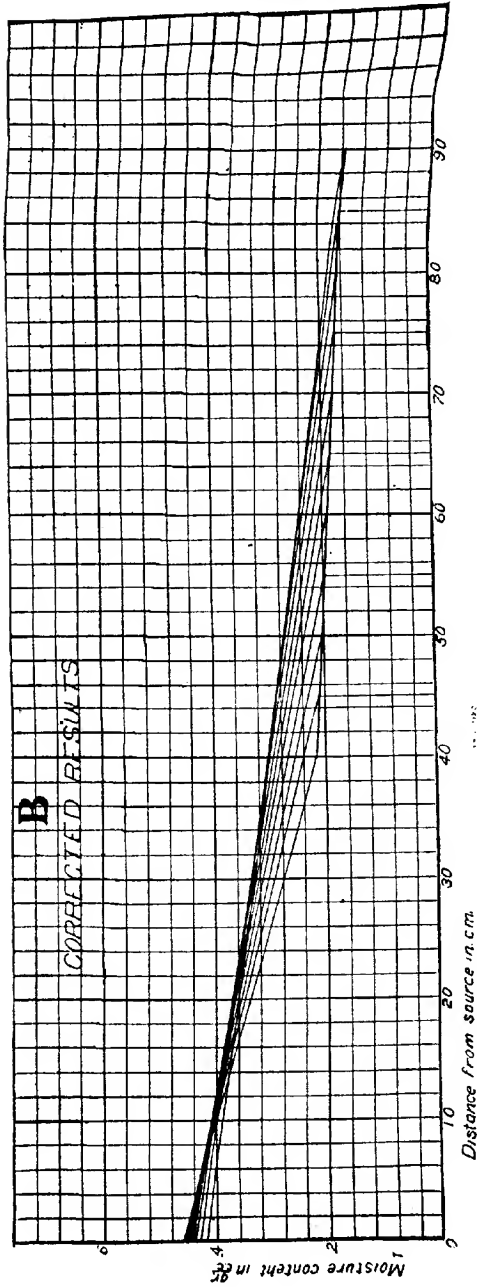
FIG. 8B

quantities which become increasingly important with increase of the time t. It is probable, however, that the somewhat arbitrary rate of irrigation may have had some influence on these figures.

TABLE 2

Values of capillary transmission constant for Greenville soil obtained from laboratory data by means of formula, $K = \dfrac{a^2}{2\phi t}$

1	2	3	4	5	6	⁺7	8	9	MEAN
5.8	5.8	6.3	5.5	5.4	6.2	5.6	5.4	5.5	
6.3	6.5	5.0	4.8	5.1	6.1	5.7	5.2	4.9	
5.2	6.6	4.7	4.7	4.9	5.4	5.1	4.8	4.7	
4.7	5.1	4.5	4.5	4.4	4.5	4.5	4.2	4.3	
4.2	4.7	4.0	4.0	4.0	4.4	3.6	3.4	3.8	
3.7	3.5	3.4	3.4	3.2	3.1	3.0	2.9		
5.0	5.2	4.6	4.4	4.5	5.0	4.6	4.3	4.6	4.7

The values obtained for this transmission function as recorded in the tables were obtained with the same soil but there were in reality four conditions of porosity involved, viz., an extremely loose unpacked soil, a soil well packed by previous wetting and drying, a soil packed dry by a systematic tapping of the tube without tamping, and a soil in the field, the latter being a case of flow under gravity and capillarity. The means for the several soil conditions are recorded in table 3.

TABLE 3

Mean values of K for Greenville soil

UNPACKED	PACKED DRY	FIELD SOIL	SOIL WELL PACKED	MEAN
-1.8×10^{-2}	-7.4×10^{-3}	-8.7×10^{-2}	-5.4×10^{-2}	-5.8×10^{-2}

The following illustrative calculation has been made on the asumption that -5.8×10^{-3} is the correct value of this constant for Greenville soil under average conditions and on the further assumption that the influence of gravity may be neglected, which should in reality be taken into account.

Problem. From a water-table 12 feet below the suface in a soil such as the Greenville soil, calculate the amount of water available at the surface.

Solution. From the formula,

$$f = \frac{-5.8\, p \times 10^{-3}}{\rho^{\frac{1}{2}}}$$

we may obtain the flow in cubic centimeters per square centimeter of surface per second of time. Let us assume the amount of moisture in grams per

cubic centimeter at the point of saturation ρ_0 to be 0.48, and the mean value of ρ 0.24. The gradient p is then obtained in c. g. s. units as follows:

$$p = \frac{0.0 - 0.48}{12 \times 30.5} = -1.31 \times 10^{-3}$$

The cube root of the mean density is 0.62. We therefore obtain

$$f = \frac{(-5.8)(-1.31)}{0.62} \times 10^{-6} = 1.2 \times 10^{-5} \text{ cc. per second.}$$

By an obvious reduction it may be seen that this would be sufficient water to cover the surface to a depth of approximately 12 inches in a period of thirty days.

It is true, of course, that caution should be exercised in the use of this constant until further experimental work has been done. It would seem, however, that there is more or less merit in the method of attack of the problem and it is hoped that other workers may become interested in this point of view. For soils high in colloidal material it may be urged that this transmission function is dependent upon the moisture content, but it does not appear that an average soil should vary appreciably under field conditions because of expansions or contractions of colloidal particles. In any event a fair solution of the problem for the ideal case should lend some assistance toward the solution of those cases which are more complex.

SUMMARY

1. A capillary transmission constant has been defined on theoretical, grounds. This constant is similar to the specific electrical conductivity of metals and to the specific thermal conductivity of heat conductors.

2. Methods have been described for the measurement of this constant in the laboratory.

3. Preliminary results have been obtained from the various methods for the Greenville soil, including values for soil under field conditions, the latter not corrected for the influence of gravity. The values obtained for this soil under various conditions of porosity range from -1.8×10^{-3} for a very loose unpacked soil to -8.7×10^{-3} for a field plot, with a mean value of -5.8×10^{-3} c. g. s. units.

4. An illustrative calculation has been made indicating that approximately 12 inches of water may be available from a 12-foot water-table in a period of 30 days. This figure has not been corrected for the influence of gravity.

5. Emphasis is laid on the general method of attack rather than upon the finality of results obtained.

that have been made from time to time, and to Dr. F. L. West, the head of the department, for coöperative encouragement during the progress of the work.

REFERENCES

(1) BRIGGS, LYMAN J.　1897　The movement and retention of water in soils.　*In* U. S. Dept. Agr. Yearbook, 1898, pp. 399–404.

(2) BRIGGS, LYMAN J.　1898　The mechanics of soil moisture.　U. S. Dept. Agr. Bur. Soils Bul. 10.

(3) BUCKINGHAM, EDGAR　1907　Studies on the movement of soil moisture.　U. S. Dept. Agr. Bur. Soils Bul. 38.

(4) CAMERON, F. K., AND BELL, J. M.　1905　Reference in Abstract 1150, Science Abstracts, Section A, v. 22, pt. 9, p. 416.　(The original article was not available to the author.)

(5) GARDNER, WILLARD　1919　The movement of moisture in soil by capillarity.　*In* Soil Sci., v. 7, no. 4, p. 313–319.

(6) SLICHTER, CHARLES S.　1898　Theoretical investigation of the motion of ground water.　*In* U. S. Geol. Survey 19th Ann. Rpt., pt. 2, p. 330.

(7) WIDTSOE, J. A.　1912　The movement of water in irrigated soils.　Utah Agr. Exp. Sta. Bul. 115, p. 217.

ORGANIC PHOSPHORUS CONTENT OF OHIO SOILS

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INTRODUCTORY

Considering the nature of the changes which are brought about in soil con-stituents through biochemical agencies, it has been the opinion of various soil investigators that an appreciable part of the soil's total supply of phosphorus is in organic combinations.

The methods used in the earlier work on this subject gave indications of a qualitative nature only, and did not approach with any degree of exactness to an accurate measure of the organic phosphorus of the soil. In fact, until recently the evidence that a significant part of the soil's supply of phosphorus is organically combined was largely circumstantial. It was assumed that the presence of an excess of phosphorus in the surface soil accompanied by an ex-cess of organic carbon and nitrogen, while potassium and other inorganic con-stituents were about the same as in the subsurface, was conclusive evidence of the presence of organic phosphorus. Other methods of estimation, such as increased solubility of phosphorus in dilute acids after ignition or treatment with oxidizing agents, have been proposed, but the indictions of some of these have been called into question, while others have perhaps never received the extended study their possibilities would warrant.

Previous to the work of Potter and Benton (4), who developed what is apparently a reliable procedure for the determination of the organic phos-phorus in ammonia extracts of soil, there has been no method for the estima-tion of any part of the organically combined phosphorus in soil which could be considered in any degree satisfactory. In a former paper (7) from this laboratory, attention has been directed to defective features in some of these earlier methods and data published which were obtained in an extended study of the method described by the authors named. The procedure neces-sary for securing the maximum possible extraction of organic phosphorus from the soil studied was described in the paper cited. The data there reported led to the conclusion that the organic phosphorus obtained in ammoniacal ex-tracts of the acid-extracted soil is a very close approximation to the soil's entire content of phosphorus so combined.

As a part of a comprehensive plan for the study of the phosphorus combina-tions in soils, and for the purpose of obtaining further information on the

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general subject of organic phosphorus in soils, it has been considered desirable to compare virgin and cultivated surface and subsurface samples from a number of representative types of Ohio soils, with respect to organic phosphorus content and such other data as may appear to be related thereto. The work done in pursuance of this plan forms the subject matter of the present paper.

SOILS STUDIED

The samples considered here are from two depths, 0–7 inches and 7–15 inches, designated "a" and "b" respectively, in the laboratory numbers. They have been selected from a number of samples taken from important soil types of the state. The points considered in their selection have included similarity in total potassium content, as indicating similarity in mineralogical composition, between the virgin and cultivated samples and the surface and subsurface. In addition, samples showing any abnormal departure from the average of the type or inconsistencies in total phosphorus or nitrogen content have not been included. Another point to which much importance was attached is similarity in reaction to litmus paper, although several exceptions to this have been admitted if otherwise suitable. The reaction of these soils, as well as the calcium, magnesium and carbonate content have been discussed at length in a former publication (1). The names applied to the soil types represented by these samples are those of the Ohio soil survey.

The history of none of the cultivated samples is known with exactitude. Sample 36 was stated by the owner to have been cultivated not less than 70 years. Numbers 19 (from a dairy farm) and 34 had been cropped for 50 years, 21 and 38 "a long time," and 32 for 30 years. It is believed that none of the cultivated soils has been under the plow for less than a generation, and so far as could be learned none had ever been limed or had received any commercial fertilizer. The virgin samples were all taken from land, usually woodland with large trees, which was known with reasonable certainty never to have been cropped. In selecting locations for samples, care was taken to secure the virgin samples in as close proximity as possible to the place where the cultivated samples were obtained and from land of similar topography, in each case typical of the soil area being sampled.

ANALYTICAL METHODS

The methods of analysis employed were essentially those described in a former paper (7). The extractions with acid and dilute ammonia were conducted as follows. Three hundred grams of ground soil were weighed into a liter Florence flask and the flask filled with approximately 1 per cent hydrochloric acid (25 cc. concentrated acid per liter), stoppered and shaken frequently for 1 hour. The contents of the flask were then shaken up and poured upon a 15-cm. Büchner funnel with two filter paper circles upon the plate.

As soon as filtered, the soil was washed with 2 liters of the 1 per cent hydrochloric acid and finally with 1 liter of saturated carbon dioxide solution, which was found sufficient to remove the hydrochloric acid in every case. Suction was employed in the filtration but care was taken not to allow the wash liquids to be drawn through too rapidly in case a very permeable sample was being filtered. The filtrates were made to 4 liters, by weight, mixed and 300-cc. aliquots taken for the determination of phosphorus removed from the soil by washing with 1 per cent hydrochloric acid. The cake of soil was transferred to a Winchester bottle, the rinsings from the funnel, etc. added, and the requisite amount of strong ammonia to make the finished solution 2.5 per cent NH_3 (150 cc.). The bottle was finally made to such weight with water that the volume of the 2.5 per cent ammonia in contact with 300 gm. of soil was 1500 cc. The two filters partially compensate for the loss in weight from acid extraction, and as none of these soils contained an undue amount of acid-soluble material, this and the slight evaporation during filtration were considered sufficient compensation. After shaking for 6 hours in a mechanical shaker 3 gm. of powdered ammonium carbonate were added and the mixtures shaken and allowed to stand some time. The contents of the bottles were finally shaken and poured into 25-cm. Büchners with two paper circles, connected by large rubber stoppers to 4-liter wide-mouth bottles. The funnels were covered with well fitting glass plates and after several hundred cubic centimeters had run through and the filtrates were free from clay, the apparatus was disconnected, the filtrates poured back and the apparatus again connected. The bottles were partially exhausted (a 25-cm. mercury vacuum was found safe) sealed and allowed to stand until the extract had passed the filter, renewing the vacuum from time to time if necessary. A very small amount of glycerine was found indispensable as a lubricant for the rubber stoppers.

The method for inorganic phosphorus in ammonia extracts has been very satisfactory with all these samples with the excepion of those representing the Brookston silty clay loam (36 and 37). In these cases all the precipitates were abnormal in appearance and behavior, and only by redissolving the ignited magnesium pyrophosphate and reprecipitating with official molybdate solution and magnesia mixture could the results of two determinations be made to agree. As the extracts of these samples gave no unusual difficulty in the total phosphorus determination, it is probable that the contaminant was partly organic. In the determination of total phosphorus in ammonia extracts by the wet combustion method, it was found that the final precipitates of magnesium ammonium phosphate from some of these samples were contaminated with iron, therefore it was necessary to resort to reprecipitation by molybdate and magnesia mixture. Although not absolutely necessary in all cases, this was always done in the work here reported.

The phosphorus in the soil was determined by the magnesium nitrate-volumetric method (2). Total nitrogen was determined by the official method (2). Ammonia-soluble organic matter (humus) and humus ash were deter-

mined by evaporating 50 cc. of the ammonia extract to dryness in a weighed platinum dish on the steam bath, drying 4 hours at 100°C. in vacuo over P_2O_5, weighing, igniting, and again weighing. No correction for combined water in the ash was applied.

Comparative color of the ammonia extracts was determined by diluting 2 cc. to 100 cc. and comparing in a Schreiner colorimeter with an extract similarly treated and arbitrarily given the value 100. It should be noted that the numbers serving as the index for comparative intensity of color vary directly with the intensity of color. Thus, an extract with the number 50 has one-half the color intensity of the standard.

SIGNIFICANCE OF ANALYTICAL DATA CONSIDERED

In table 1, the analytical data for these soils, of interest in the present connection, are presented. These include total phosphorus in the soil, inorganic and organic phosphorus in ammonia extracts and phosphorus removed from the soil by washing with 1 per cent hydrochloric acid and saturated carbon dioxide solution in preparing the samples for extraction with ammonia solution.

While the direct connection between the acid-soluble and the organic phosphorus content of the soil may not be apparent, the amount of phosphorus removed from the soil by leaching with dilute hydrochloric acid is considered to be of some significance in a soil study of this nature, for the reason that it may serve to indicate variations in the state of combination of soil phosphorus or serve as an index to the more readily soluble or presumably more available part of the total phosphorus, as other procedures of extraction with dilute acids have been supposed to do. It should be understood that the hydrochloric-acid leaching removes much more phosphorus from the soil than does a single extraction with fifth-normal nitric acid, which has been used to a considerable extent for this purpose in the past. This is true, not so much because one is a more powerful solvent than the other, but because a process of leaching tends to reduce to a minimum the reabsorption of phosphorus once dissolved. This phase of the subject has been studied at length by Prescott (6).

From data published in a former paper (7), it appeared that in the case of the soil then studied, inorganic phosphorus absorbed by the soil from acid solution was completely recovered as inorganic phosphorus in water washings and a subsequent ammonia extraction. Unfortunately, no work intended to confirm this point for the soils now under consideration has been done, so that in these cases there is no proof that the absorbed phosphorus is completely removed by a subsequent ammonia extraction. The most that can be said is that the phosphorus neither removed by acid leaching nor appearing in the ammonia extract is about the maximum amount which can be considered to be in a form very resistant to solvents, possibly because it is enclosed in mineral particles. If this last statement is true, it seems probable that there

would be evident, in many cases, a certain uniformity among figures so obtained for virgin and cultivated surface and subsurface soils of the same type, of similar origin and mineralogical composition.   Such being the case, it would indicate that the figures for organic phosphorus are probably not grossly inaccurate by reason of incomplete extraction at least, since it is improbable that the surface and subsurface samples would contain the same amounts of organic phosphorus not extracted by ammonia.   This phase of the subject will be dismissed with the statement that the percentages of insoluble phosphorus, calculated on the soil, do in most cases show a marked similarity. The variation between soil types is large, but several types show nearly as large differences between surface and subsuface of the same sample.   As the percentages can readily be calculated from other data in table 1, they are not tabulated.

The total nitrogen contents of these samples are included, because these serve as indicators of the relative amounts of organic matter in the samples. The comparative color, total organic matter (humus) and humus ash in the ammonia extracts are included to establish their relation to the content of organic phosphorus.

DISCUSSION OF ANALYTICAL DATA

In nearly all cases the virgin surface sample contains more total phosphorus than the corresponding cultivated sample.   The same is true of the subsurface samples also, although here the average difference is comparatively small.

The ammonia-soluble organic phosphorus of both depths averages higher with virgin samples than with cultivated.   In each depth, the average percentage of the total phosphorus which is in the ammonia-soluble organic form is practically the same in the cultivated samples as it is in the virgin samples. One-third the total phosphorus of the average surface sample is organically combined, while in the subsurface samples the average proportion is one-fifth. As it happens, the extremes are in the cases of the two sandy soils, the Dunkirk fine sand having but 18 and 20 per cent of the total phosphorus in the surface depths of the cultivated and virgin samples, respectively, in the organic form, while the Clyde fine sand shows 52 and 50 per cent so combined in the corresponding samples.   The lowest proportion of total phosphorus in organic form in the case of a subsurface sample is found in the case of the virgin Wooster silt loam, 6 per cent; the highest is 47 per cent in the virgin Clyde fine sand.

The proportion of the total phosphorus occurring as ammonia-soluble inorganic averages slightly higher in the subsurface than in the surface samples. The average figure is 11 per cent for both virgin and cultivated samples of this depth, but 8 and 9 per cent, respectively, for surface samples.   The extremes are 2 per cent in the subsurface of the cultivated Clyde fine sand and the surface of the virgin Crosby silt loam and 20 per cent in the subsurface of the vir-

TABLE 1

Data on soils investigated

DESCRIPTION	NUMBER	PHOSPHORUS							TOTAL NITROGEN	COMPARATIVE COLOR OF NH₄OH EXTRACTS	NH₄OH-SOLUBLE ORGANIC MATTER (HUMUS)	HUMUS ASH	ORGANIC P IN NH₄OH-SOLUBLE ORGANIC MATTER	REACTION OF SOIL
		Total	NH₄OH-soluble organic	Organic as per cent of total	NH₄OH-soluble inorganic	NH₄OH-soluble inorganic as per cent of total	HCl-washing-soluble	HCl-washing-soluble as per cent of total						
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent		per cent	per cent	per cent	
Wooster loam......... Cultivated	4a	0.0530	0.0165	31	0.0045	8	0.0062	12	0.14	63	1.430	0.197	1.15	Acid
	4b	0.0361	0.0058	16	0.0013	4	0.0015	4	0.06	18	0.444	0.213	1.30	Acid
Virgin	5a	0.0923	0.0210	23	0.0110	12	0.0339	37	0.16	89	1.691	0.170	1.24	Acid
	5b	0.0300	0.0038	13	0.0028	9	0.0043	14	0.04	11	0.309	0.168	1.21	Acid
Wooster silt loam...... Cultivated	8a	0.0342	0.0108	31	0.0050	15	0.0028	8	0.12	52	1.048	0.169	1.03	Acid
	8b	0.0307	0.0060	20	0.0033	11	0.0011	4	0.07	19	0.551	0.108	1.09	Acid
Virgin	9a	0.0520	0.0140	27	0.0063	12	0.0072	14	0.16	83	1.454	0.111	0.96	Acid.
	9b	0.0348	0.0020	6	0.0063	18	0.0021	6	0.05	8	0.310	0.090	0.65	Acid
Cincinnati silt loam...... Cultivated	19a	0.0613	0.0182	30	0.0056	9	0.0015	2	0.11	67	1.083	0.130	1.69	Acid
	19b	0.0541	0.0038	7	0.0093	17	0.0014	3	0.06	9	0.355	0.069	1.06	Acid
Virgin	20a	0.0657	0.0202	31	0.0070	11	0.0062	9	0.15	91	1.403	0.171	1.44	Acid
	20b	0.0534	0.0045	8	0.0095	18	0.0045	8	0.06	11	0.384	0.070	1.17	Acid
Clermont silt loam...... Cultivated	21a	0.0437	0.0125	29	0.0048	11	0.0010	2	0.10	28	0.870	0.412	1.44	Acid
	21b	0.0288	0.0040	14	0.0023	8	0.0010	3	0.04	5	0.352	0.470	1.14	Acid
Virgin	22a	0.0464	0.0140	30	0.0040	9	0.0016	3	0.14	40	1.247	0.359	1.12	Acid
	22b	0.0294	0.0045	15	0.0025	9	0.0008	3	0.06	9	0.377	0.484	1.19	Acid

Soil type		No.													Reaction
Crosby silt loam...........	Cultivated	34a	0.0312	0.0120	38	0.0018	6	0.0037	12	0.14	113	1.617	0.106	0.74	Acid
		34b	0.0221	0.0053	24	0.0020	9	0.0017	8	0.06	28	0.576	0.184	0.91	Acid
	Virgin	35a	0.0522	0.0148	28	0.0013	2	0.0042	8	0.17	129	1.920	0.97	0.77	Acid
		35b	0.0242	0.0058	24	0.0008	3	0.0007	3	0.07	34	0.538	0.135	1.07	Acid
Fox silt loam..........	Cultivated	59a	0.0442	0.0155	35	0.0050	11	0.0024	5	0.11	52	1.091	0.093	1.42	Acid
		59b	0.0472	0.0075	16	0.0063	13	0.0031	7	0.07	23	0.522	0.087	1.44	Acid
	Virgin	60a	0.0524	0.0228	43	0.0048	9	0.0032	6	0.18	85	1.676	1.155	1.36	Alkaline
		60b	0.0451	0.0125	28	0.0040	9	0.0026	6	0.11	48	0.909	0.097	1.38	Alkaline
Dunkirk fine sand.........	Cultivated	25a	0.0337	0.0068	18	0.0040	11	0.0084	22	0.10	63	1.169	0.085	0.58	Neutral
		25b	0.0200	0.0038	19	0.0025	13	0.0035	18	0.05	21	0.475	0.080	0.79	Neutral
	Virgin	26a	0.0318	0.0065	20	0.0020	6	0.0024	8	0.10	66	1.147	0.061	0.57	Acid
		26b	0.0211	0.0025	12	0.0013	6	0.0011	5	0.04	11	0.371	0.056	0.67	Acid
Clyde (Maumee) fine sand...	Cultivated	28a	0.0305	0.0160	52	0.0020	7	0.0053	17	0.16	250	2.356	0.345	0.68	Acid
		28b	0.0284	0.0088	31	0.0007	2	0.0025	9	0.06	68	0.820	0.303	1.07	Acid
	Virgin	29a	0.0561	0.0283	50	0.0032	6	0.0071	13	0.19	185	2.127	0.684	1.33	Acid
		29b	0.0353	0.0165	47	0.0023	7	0.0048	14	0.08	70	0.929	0.244	1.78	Acid
Miami silty clay loam.......	Cultivated	32a	0.0265	0.0095	36	0.0033	12	0.0020	8	0.12	54	1.136	1.195	0.84	Alkaline
		32b	0.0259	0.0043	17	0.0040	15	0.0017	7	0.08	14	0.475	0.129	0.90	Acid
	Virgin	33a	0.0372	0.0138	37	0.0045	12	0.0060	16	0.18	122	2.097	0.285	0.66	Alkaline
		33b	0.0317	0.0063	20	0.0043	14	0.0021	7	0.08	24	0.614	0.129	1.02	Alkaline

TABLE 1—Continued

DESCRIPTION		NUMBER	PHOSPHORUS							TOTAL NITROGEN	COMPARATIVE COLOR OF NH₄OH EXTRACTS	NH₄OH-SOLUBLE ORGANIC MATTER (HUMUS)	HUMUS ASH	ORGANIC P IN NH₄OH-SOLUBLE ORGANIC MATTER	REACTION OF SOIL
			Total	NH₄OH-soluble organic	Organic as per cent of total	NH₄OH-soluble inorganic	NH₄OH-soluble inorganic as per cent of total	HCl-washing-soluble	HCl-washing-soluble as per cent of total						
			per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent		per cent	per cent	per cent	
Lucas silt loam	Cultivated	38a	0.0448	0.0125	28	0.0030	7	0.0010	2	0.13	80	1.161	0.134	1.08	Alkaline
		38b	0.0331	0.0050	15	0.0058	18	0.0007	2	0.06	19	0.455	0.293	1.10	Alkaline
	Virgin	39a	0.0437	0.0118	27	0.0050	11	0.0054	12	0.14	82	1.226	0.202	0.96	Alkaline
		39b	0.0407	0.0080	20	0.0080	20	0.0058	14	0.08	51	0.756	0.326	1.06	Alkaline
Brookston silty clay loam	Cultivated	36a	0.0612	0.0270	44	0.0045	7	0.0061	10	0.20	165	2.224	0.170	1.21	Alkaline
		36b	0.0505	0.0188	37	0.0053	11	0.0038	8	0.12	77	1.103	0.180	1.70	Alkaline
	Virgin	37a	0.1145	0.0535	47	0.0050	4	0.0261	23	0.39	215	3.833	0.211	1.39	Alkaline
		37b	0.0698	0.0235	34	0.0050	7	0.0229	33	0.23	96	1.208	0.139	1.94	Alkaline
Newton loam	Cultivated	49a	0.0509	0.0198	39	0.0033	6	0.0191	37	0.24	214	2.678	0.513	0.74	Alkaline
		49b	0.0368	0.0068	18	0.0033	9	0.0174	47	0.10	67	0.880	0.625	0.77	Alkaline
	Virgin	50a	0.0604	0.0258	43	0.0023	4	0.0220	36	0.28	231	3.351	0.295	0.77	Alkaline
		50b	0.0421	0.0100	24	0.0028	7	0.0209	50	0.10	59	1.000	0.597	1.00	Alkaline
Average of 12 soil types	Cultivated	0-7	0.0433	0.0148	34	0.0039	9	0.0050	11	0.14	100	1.489	0.212	1.05	
		7-15	0.0345	0.0067	20	0.0038	11	0.0033	10	0.07	31	0.584	0.228	1.11	
	Virgin	0-7	0.0587	0.0205	34	0.0047	8	0.0104	15	0.19	118	1.931	0.233	1.05	
		7-15	0.0381	0.0083	21	0.0041	11	0.0061	14	0.08	36	0.642	0.211	1.18	

gin Lucas silt loam. It should be noted that the figure for ammonia-soluble inorganic phosphorus is to some extent influenced by the technique of the previous acid extraction, so that these data are of uncertain significance.

The percentages of the total phosphorus removable by leaching with 1 per cent hydrochloric acid and washing with a saturated solution of carbon dioxide are on the average distinctly higher in the cases of both depths of virgin samples. This is in harmony with the results obtained for fifth-normal-nitric-acid-soluble phosphorus and is doubtless to be attributed to depletion of the more soluble forms of phosphorus as the result of cultivation. In the cases of several types, however, the cultivated sample contains the greater amount of acid-soluble phosphorus.

As might be expected, the data for total nitrogen show very distinctly the effect of cultivation upon the surface soil. The effect upon the subsurface is much less, in fact several samples show as much total nitrogen in the lower depth of a cultivated sample as in the virgin sample of the same depth, or still more. In general, within each type there is apparent a marked relation between total nitrogen and the ammonia-soluble organic matter, organic phosphorus and color. The Clyde fine sand is rather exceptional in the lack of such correlation. In comparing different types a similar, though commonly less exact relation between these constituents is observed. From the averaged data for all types, it was found that the ratio is such that if the ammonia-soluble organic matter of the surface soil is represented by 100, the total nitrogen will approximate to 10 and the organic phosphorus 1. For the subsurface samples, the ratio of soil nitrogen to organic phosphorus is slightly higher, and the relative amount of ammonia-soluble organic matter is somewhat less.

The percentage relations of the ammonia-soluble organic phosphorus to humus are included in table 1. From these, it may be observed that the least proportion of organic phosphorus to organic matter in ammonia solution is found in the case of the surface soil of the Dunkirk fine sand; the figure here is slightly less than 0.6 per cent for both samples, and the subsurface samples also show percentages but a trifle greater. The highest percentage of organic phosphorus in ammonia-soluble organic matter is found in the case of the virgin subsurface of the Brookston silty clay loam, the figure being 1.94 per cent. The percentage of organic phosphorus in the ammonia-soluble organic matter is distinctly higher in the case of the subsurface samples and in most cases the organic matter from the subsurface of virgin samples contains more than that from cultivated samples.

The comparative color of ammonia extracts is more nearly directly proportional to the organic matter in the extract than to organic phosphorus or total nitrogen in the soil. Considering the amount of the color due to ferric hydroxide, it could scarcely be expected to show any very close relations to other constituents of the solution.

The percentages of humus ash contained in the ammonia extracts of these soils do not exhibit any close relation to other constituents found in solution.

In general, it may be said that surface soils containing much organic matter soluble in ammonia run higher than the average in humus ash. Subsurface samples, however, sometimes furnish an extract with more ash than that from the corresponding surface soils. The percentage of ash in the ammonia extract is to a large extent dependent upon the effectiveness of the filtration procedure. It was found in a previous investigation (7) that the method of filtration employed for these samples affords an extract practically free from clay. With most samples ferric oxide is the predominant constituent of the ash.

REACTION OF SOIL AND ORGANIC PHOSPHORUS CONTENT

In so far as the data obtained permit any opinion to be formed, reaction of the soil is without influence upon the organic phosphorus. The soils included in this investigation are predominantly acid; there are but four types in which both cultivated and virgin samples are alkaline. Of these four types, the Miami silty clay loam and the Lucas silt loam are upland soils and the Brookston silty clay loam and Newton loam are representative of the dark-colored, often poorly drained neutral or alkaline soils so common in the northwestern quarter of the state, and formerly referred to the Clyde series of soils on account of their high content of organic matter, which is the distinguishing characteristic of the Clyde series. The soils first named are rather below the average of all the types here considered in total nitrogen, ammonia-soluble organic matter and organic phosphorus, while the second pair of soils are distinctly above the average in all these constituents.

The averaged percentages of organic phosphorus in ammonia-soluble organic matter of these soils are not markedly different from those of the other types considered, nor are the ratios of these constituents to total nitrogen dissimilar when averaged for the four types, although including samples which are near extremes in both directions with respect to ratios of nitrogen, humus and organic phosphorus.

It is true that the dark-colored alkaline soils under discussion are higher than the average in total phosphorus, and that an unusually large proportion of this is present in the organic form. But the Clyde fine sand, although lower in total phosphorus, has an even larger proportion of this in the organic state, and this soil is acid. The only relation existing between reaction and organic phosphorus content seems to follow from the fact that the chief factor favoring the accumulation of organic matter (and organic phosphorus with it) is poor drainage, and this also operates toward the conservation of bases, the soil remaining alkaline if it was well supplied with basic material at the beginning.

CULTIVATION, SOIL REACTION AND NATURE OF ORGANIC PHOSPHORUS

The data in table 1 show that the organic phosphorus content of virgin surface soils is in general considerably greater than that of the corresponding cultivated samples; it has just been said that the reaction of the soil appears

to be without marked influence upon its content of organic phosphorus. The question of the influence of these factors upon the nature of the organic phosphorus compounds of the soil remains to be answered. As a means for detecting any difference in composition which might exist between the organic phosphorus compounds of different soils, the procedure of hydrolysis with 5 per cent sulfuric acid, as used by Jones (3) in his studies of the nucleotides and by Potter and Snyder (5) for ammonia extracts of soil, seemed promising.

The experiment was conducted in the following manner. Eight-hundred-cubic-centimeter portions of the ammonia extracts from the surface soil, both virgin and cultivated, of the pronouncedly acid Wooster loam, the very slightly acid or nearly neutral Clermont silt loam and the alkaline Newton loam were

TABLE 2

Decomposition of organic phosphorus compounds by boiling 5 per cent H₂SO₄

TIME OF HEATING	SAMPLE 4A			SAMPLE 21A			SAMPLE 49A		
	Inorganic P found	Increase due to hydrolysis	Per cent of organic P decomposed	Inorganic P found	Increase due to hydrolysis	Per cent of organic P decomposed	Inorganic P found	Increase due to hydrolysis	Per cent of organic P decomposed
hours	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*
0	2.2	(0.8*)	(15)	1.8	(0.3*)	(8)	1.5	(0.5*)	(8)
½	2.6	0.4	9	2.2	0.4	11	1.8	0.3	5
1:05	2.9	0.7	16	2.3	0.5	14	2.1	0.6	10
2	3.1	0.9	20	2.5	0.7	19	2.4	0.9	15
4	3.3	1.1	24	2.7	0.9	24	2.9	1.4	24
	SAMPLE 5A			SAMPLE 22A			SAMPLE 50A		
0	4.5	(1.0*)	(15)	2.3	(1.0*)	(22)	1.9	(1.2*)	(14)
½	4.8	0.3	5	2.5	0.2	6	2.1	0.2	3
1	4.9	0.4	7	2.6	0.3	9	2.5	0.6	8
2	5.1	0.6	11	2.8	0.5	14	2.8	0.9	13
3¾	5.6	1.1	19	3.1	0.8	23	Lost		

* Increase over that contained in the original solution, due to decomposition during preparation for experiment, including momentary boiling.

allowed to stand in large porcelain dishes for several days, in a place protected from dust but exposed to a constant current of air, until the ammonia had evaporated and the volume was reduced about one-half. The solutions were then transferred to 500-cc. flasks and made to volume with washings from the dishes. Aliquots of 100 cc. were transferred to 200-cc. extraction flasks, sufficient dilute sulfuric acid added to make the solution 5 per cent by weight and reflux condensers attached. The flasks were set, three at a time, upon an electric hot plate and heated as rapidly as possible to boiling, each time starting with the plate cold. After the solutions had boiled the stated time, the flasks were immediately cooled with water, the contents poured into centrifuge bottles, made alkaline with ammonia and treated in the usual manner for the

inorganic phosphorus determination. The results obtained are presented in table 2, also in graphic form in figure 1.

X - 4a   △ - 21a   ☐ - 49a   Cultivated ————
⊗ - 5a   ⊕ - 22a   ⊘ - 50a   Virgin. ‑‑‑‑‑‑‑
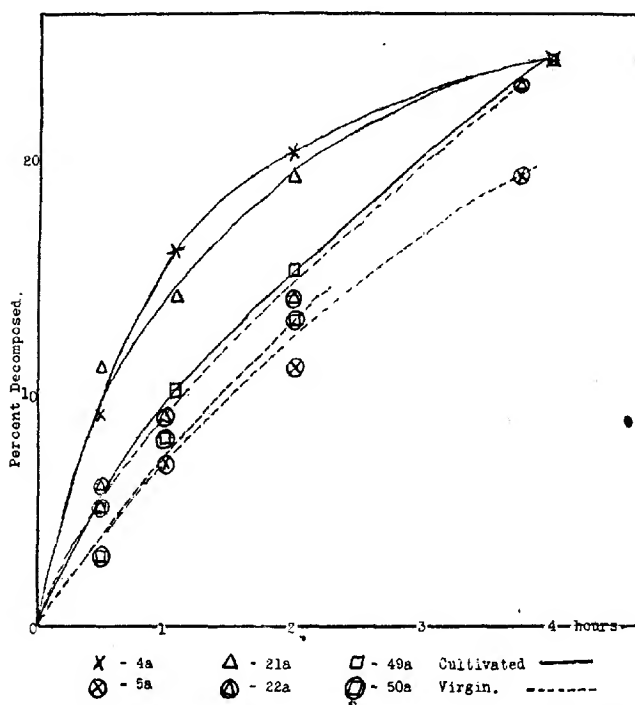
FIG. 1. HYDROLYSIS OF ORGANIC PHOSPHORUS COMPOUNDS BY 5 PER CENT H_2SO_4 AT BOILING TEMPERATURE

In explanation of table 2, it should be recalled that the samples represent 160 cc. of the original extract, and the original contents of inorganic and organic phosphorus were as follows:

SAMPLE	INORGANIC PHOSPHORUS	ORGANIC PHOSPHORUS
	mgm.	*mgm.*
4a	1.4	5.3
5a	3.5	6.7
21a	1.5	4.0
22a	1.3	4.5
49a	1.1	6.3
50a	0.7	8.3

The figures in table 2, enclosed in parentheses, represent increases over the corresponding original amounts of inorganic phosphorus as given above, and the percentage relation of the same to the original organic phosphorus contents. These indicate the amount of decomposition during preparation of the solutions for the experiment, including momentary heating to boiling temperature with immediate cooling, and serve as blank determinations with the time of hydrolysis at zero. Elsewhere in table 2, the amounts of inorganic phosphorus found after boiling for a stated time have been reduced by the corresponding quantities to which reference has been made and the percentages of decomposition have been calculated on the basis of the organic phosphorus remaining undecomposed after this preliminary treatment. These percentages of decomposition are plotted as ordinates in figure 1, while the periods of boiling, in hours, are abscissae.

The data presented in table 2 and plotted in figure 1 indicate that the organic phosphorus compounds of all the cultivated soils are somewhat less resistant to hydrolysis than are those of the virgin soils. It is noteworthy that decomposition during 4 hours' boiling has been precisely the same in the cases of all the cultivated soils, 24 per cent, although the percentages at intermediate periods do not coincide. Of the cultivated samples, the curves representing the acid Wooster loam, 4a, and the slightly acid Clermont silt loam, 21a, show considerable resemblance. Among the virgin samples, the Wooster loam, 5a, shows the greatest divergence from the other two, and also the greatest difference between a virgin and cultivated sample. The alkaline Newton loam shows greatest resemblance between data for virgin and cultivated samples, possibly because its extracts contain most organic phosphorus and any percentage errors are thus smaller. The results of this experiment are so indecisive that extended comment does not seem to be warranted. Neither striking resemblances nor marked dissimilarity among the organic phosphorus compounds of virgin and cultivated soils, both acid and alkaline, is indicated.

AVAILABILITY OF PHOSPHORUS IN ORGANIC COMPOUNDS

While it was not intended to include in this study any reference to the question of the amount of phosphorus supplied to vegetation from that stored up in the organic form, this being a subject for more extended investigation, it may be well to direct attention to some facts disclosed by the data obtained and which seem to furnish indications regarding this phase of the subject. The general relation found to exist between the humus, total nitrogen and organic phosphorus of the soils investigated and the marked similarity of the ratios between these constituents in virgin and long cultivated soils of the same type has been pointed out. The considerable resistance to decomposition by hydrolysis with boiling 5 per cent sulfuric acid shown by the organic phosphorus compounds of all the samples in which this was determined, has been demonstrated. The ready response to phosphorus fertilization shown by

practically all the soils of the state on which fertility experiments have been conducted has been shown by the work of the Ohio Station. Considered together, these facts do not indicate that the phosphorus in organic combinations becomes available very rapidly, certainly not at a generally greater rate than the organic nitrogen associated with it in the soil. Further, it seems altogether probable that the various agencies, including liming and frequent cultivation, known to result in a more rapid utilization of the soil's store of nitrogen, will have a similar effect upon the phosphorus in organic combinations.

SUMMARY

A study of the organic phosphorus content of samples from 12 representative types of Ohio soils, and the relation of the same to other soil constituents, is reported. The observations made have been based upon the examination of virgin and cultivated samples, surface and subsurface, from each soil type considered.

Averaged figures indicate that virgin surface samples are considerably richer in total phosphorus than the corresponding cultivated samples of the same type, and that virgin subsurface samples contain slightly more total phosphorus than cultivated soils at the same depth.

The organic phosphorus contents of the several samples from the average soil type stand in the same order as the contents of total phosphorus.

The organic phosphorus bears very nearly the same percentage relation to the total phosphorus in cultivated soils as in virgin soils at the same depths, in the greater number of cases.

From averaged data, one-third the phosphorus in the surface and one-fifth that in the subsurface samples of both virgin and cultivated soils is in the organic form.

The organic phosphorus and humus soluble in ammonia are shown to be closely related to each other, to total nitrogen in the soil and, to a less extent, the color of the ammonia extract. From averaged data, if 100 represents the percentage of ammonia-soluble humus obtained from a soil, the total nitrogen in the soil is 10, and the organic phosphorus in the ammonia extract is 1. Except as noted, there does not appear to be any connection between other soil constituents and organic phosphorus content.

Reaction of the soil appears to be without influence upon the quantity and nature of the organic phosphorus present.

There is some evidence that the organic phosphorus compounds of cultivated soils are decomposed slightly more readily than are those of the virgin soils examined.

From general considerations, it is thought that the phosphorus in organic combinations in the soil is not of a very high order of availability.

REFERENCES

(1) AMES, J. W., AND SCHOLLENBERGER, C. J. 1919 Calcium and magnesium content of virgin and cultivated soils. *In* Soil Sci., v. 8, p. 323–335.

(2) Association of Official Agricultural Chemists 1916 Committee on Editing Tentative and Official Methods of Analysis. Williams & Wilkins Co., Baltimore.

(3) JONES, W. 1916 An indirect method of determining pyrimidine groups in nucleotides. *In* Jour. Biol. Chem., v. 24, no. 3, p. 3–10.

(4) POTTER, R. S., AND BENTON, T. H. 1916 The organic phosphorus of soil. *In* Soil Sci., v. 2, p. 291–298.

(5) POTTER, R. S., AND SNYDER, R. S. 1918 The organic phosphorus of soil. *In* Soil Sci., v. 6, p. 321–332.

(6) PRESCOTT, J. A. 1916 The phenomena of adsorption in its relation to soils. *In* Jour. Agr. Sci., v. 8, pt. 1, p. 111–130.

(7) SCHOLLENBERGER, C. J. 1918 Organic phosphorus of soil: Experimental work on methods for extraction and determination. *In* Soil Sci., v. 6, p. 365–395.

THE EFFECT OF THE INITIAL MOISTURE IN A SOIL ON MOISTURE MOVEMENT

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A review of the literature on the effect of initial moisture on moisture movement has been given by Alway and McDole (1). Their statement in this connection is, "No definite conclusions are to be drawn as to the effect of the initial moistness upon movement of water."

Experimental work of their own is also reported. "Seventeen soils, ranging from a coarse sand with a hygroscopic coefficient of 0.6 to a silt loam with one of 13.3, were placed in cylinders in three different degrees of moistness, 0.5, 1.0, and 1.5 times the hygroscopic coefficient, 1 inch of water was applied and the rate of movement during 5 days observed." The percentage of water in the moistened portions at the various times also was determined. It was found that the distance of penetration increased with increase in initial moisture, but that the percentage of moisture in the moistened portions of the soils was no greater, and usually smaller, in the soils of lower initial moistness. The fact that the distance of penetration was less in soils of low initial moistness was evidently due not to a less favorable condition for capillary movement in these soils ahead of the advancing water layer, but to the stronger force with which the smaller amount of water was held within the moistened portion. Had penetration been determined in the presence of a constant supply of gravitational water, no doubt it would have been independent of the initial moisture condition.

Glass tubes were also filled with the same soils in the same three degrees of moistness and the lower ends kept in contact with water at a constant level. Movement up into the soils was generally most rapid in the soils of highest initial moisture, but slowest in those of intermediate moisture condition, so that "no definite dependence of rise upon initial moistness was shown."

Shortly after the date of the above publication, Harris and Turpin reported work on moisture movement, dealing in part with the effect of initial moistness in this connection (3). Their results show that, on the whole, distance of movement is greater in soils of higher initial moistness, particularly when sufficient time is not given for the establishment of entire equilibrium in all soils. Again, however, there is no evidence from their published data of a less favorable condition for penetration of capillary moisture into soils of low initial moistness. The percentage of moisture in the moistened portions of

these soils never increased above that in the moistened portions of the soils of high initial moistness, as would be expected if the dry soil condition presented less favorable conditions for the penetration of capillary moisture than the moist soil condition.

EXPERIMENTAL WORK

Some work was done by the writer at the Michigan Agricultural College during the years 1913 and 1914, on the effect on moisture movement of changes in the surface tension of the soil solution brought about by the addition of soluble salts (4). The salts were mixed with portions of soils. These were then put in short tubes, saturated, and, after percolation ceased, placed in capillary connection with a dry soil underneath. The effect of the salts was determined by weighing from time to time and noting the relative rates at which the differently treated soils gave up water to the soil underneath. The question arose in this connection of the reflex effect on movement of water from the tube soils of differences in the degree of moistening of the soil underneath; for instance, would a tube soil which naturally gave up its moisture slowly be further handicapped on account of the slowness of the moistening of the soil underneath?

A number of laboratory experiments made at the time with two soils—a sandy loam and a clay loam—showed no appreciable effect of differences in initial moistness on moisture movement of water subsequently added. Some of these results, hitherto unpublished, are as follows:

Experiments with sandy loam soil

1. Soil brought to desired initial moisture conditions before being placed in tubes (glass tubes for the most part 2½ inches in diameter, were used in these experiments); same amount of packing given to the two soils; water added to the bottom of the tubes. Movement of water through soil aided by head of water of about 20 cm.

2. Same procedure as in (1) except that the moist soil was packed to a degree to give practically the same apparent specific gravity as that of the air-dry soil.

3. Water added to tops of tubes. A constant head of ¾ inch maintained. Same amount of packing given the two tubes.

Experiments with clay loam soil

1. Water added to tops of tubes, constant head of ¾ inch maintained, soils brought to desired initial moisture content before being placed in tubes.

2. Water added to tops of tubes. Constant head of ¾ inch maintained. All tubes filled with soil of high initial moisture content. Tubes for movement in dry soil reduced to approximately air-dry condition after filling. This procedure was adopted to avoid differences in packing brought about by filling with soils of different initial moisture contents.

With one exception, movement in the air-dry soils was as rapid as or more rapid than in the soils of higher initial moisture content. The exception was a clay loam tube in the first experiment with this soil, where the more rapid movement is probably to be attributed to favorable structural conditions.

TABLE 1

Experiment 1, sandy loam

TIME	DISTANCE REACHED BY MOISTURE MOVEMENT IN	
	Soil containing 5.01 per cent initial moisture	Soil air-dry, 1.00 per cent initial moisture
10 minutes...................................	6.3 cm.	6.8 cm.
35 minutes...................................	11.0 cm.	11.7 cm.
65 minutes...................................	14.7 cm.	15.0 cm.
Total water taken up.........................	272.0 gm.	306.0 gm.

TABLE 2

Experiment 2, sandy loam soil

TIME	DISTANCE REACHED BY MOISTURE MOVEMENT IN	
	Soil containing 5.94 per cent initial moisture	Soil air-dry, 1.00 per cent initial moisture
10 minutes...................................	5.2 cm.	5.5 cm.
30 minutes...................................	9.5 cm.	10.0 cm.
60 minutes...................................	13.3 cm.	13.6 cm.
Total water taken up.........................	290.0 gm.	310.0 gm.

TABLE 3

Experiment 3, sandy loam soil

TIME	DISTANCE REACHED BY MOISTURE MOVEMENT IN	
	Soil containing 6 per cent initial moisture	Soil air-dry, 1.00 per cent initial moisture
10 minutes...................................	4.4 cm.	5.0 cm.
30 minutes...................................	8.7 cm.	9.2 cm.
60 minutes...................................	12.8 cm.	13.0 cm.
Total water taken up.........................	150.0 gm.	178.0 gm.

TABLE 4

Experiment 1, clay loam soil

TIME	DISTANCE REACHED BY MOISTURE MOVEMENT IN			
	Soil containing 8.5 per cent initial moisture		Soil air-dry, 3.5 per cent initial moisture	
	Same amount of packing as air-dry tubes	Packed to give same apparent specific gravity as air-dry tubes	Both tubes packed the same	
minutes	*cm.*	*cm.*	*cm.*	*cm.*
10	5.0	4.5	4.1	3.9
30	9.5	8.0	7.6	7.4
60	13.4	10.9	10.6	10.5
90	16.4	13.1	12.9	12.8

Results with the sandy loam soil showed also a larger amount of water taken up by the soil in the air-dry condition than with the higher initial moistness.   On the whole, these results do not indicate any gain in capillary movement in soil due to higher initial moistness.

In the absence of work of a conclusive nature on this point, it seemed desirable to make additional and more accurately controlled experiments along the line of those reported above.[1]

A difficulty in determining the effect of initial moistness on movement of water added is that, in placing the soil of different moisture contents in the tubes, differences in packing result which probably are an important factor in determining the movement secured.   To avoid this, specially devised tubes were made of galvanized iron with a portion the entire length removed and a glass plate substituted (fig. 1).   This made possible the filling of the tubes with soil of the higher initial moisture contents and, by removing the glass plates and drying, the bringing about of differences in initial moisture content without changing the structural condition.   The glass plates also made possible the observation of the distance of penetration of the water film.

TABLE 5

Experiment 2, clay loam soil

TIME	DISTANCE REACHED BY MOISTURE MOVEMENT IN			
	Moist tubes, 6 per cent water added to air-dry soil		Air-dry tubes, 3.5 per cent moisture	
minutes	*cm.*	*cm.*	*cm.*	*cm.*
10	5.0	5.0	4.8	4.5
30	8.0	8.1	7.6	7.5
60	11.2	12.2	11.0	10.8
90	13.6	13.8	14.4	14.2

Four soils were used: a sandy loam, two silt loams, and a clay loam.   One of the silt loams was obtained on the station farm at Lexington and will be designated as Station silt loam; the other came from the Purchase Region of western Kentucky, a soil of loessal origin, which will be designated as Purchase silt loam.   All four of the soils were surface soils.

The hygroscopic coefficients of these soils are: sandy loam, 2.90; Station silt loam, 5.26; Purchase silt loam, 3.80; clay loam, 7.72.

The soils were used in the following initial moisture conditions: oven-dry, air-dry, hygroscopic coefficient, $1\frac{1}{2}$ times the hygroscopic coefficient, and, in the case of the sandy loam and Purchase silt loam, 2 times the hygroscopic coefficient.

Moisture movement was determined by placing the tubes in a vertical position with their lower ends in contact with water at a constant level and noting the height of rise of water from time to time.

[1] The experiments reported in the remainder of this publication were carried out in the soil laboratory of the Kentucky Experiment Station.

The results are shown in part in tables 6, 7, 8 and 9.   Considerable preliminary work was done before the final work reported in the tables.   Since the results from this work are in practical agreement with those from the later work, it does not seem necessary to include them.   The initial moisture condition of the soil, as well as how it was secured, is shown in the proper headings in the tables.   The distance of moisture movement at various times and the final total amount of water taken up are given.   The initial percentage of water in the soils as determined by drying in the oven and the apparent specific gravity of the soils also are shown.   It will be observed that the
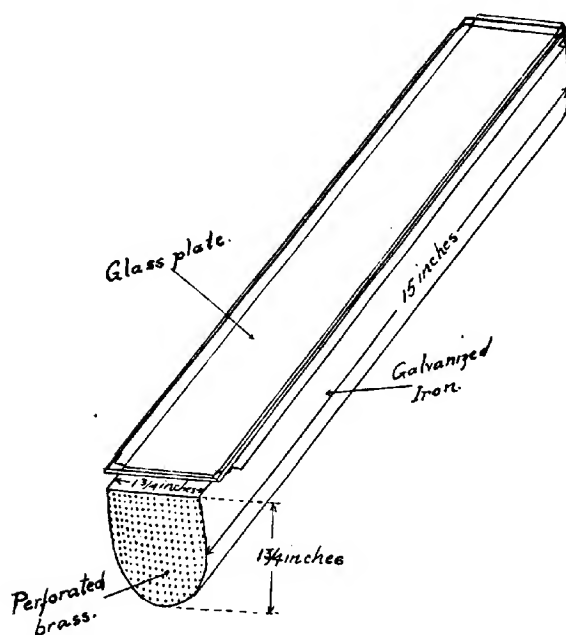
FIG. 1. DIAGRAM OF TUBES ESPECIALLY DEVISED FOR USE IN MOISTURE-MOVEMENT WORK

actual initial moisture condition always varied somewhat from the one desired. Thorough distribution of the initial water added to the soils was secured before putting them into the tubes.   The soils were packed in the tubes by adding in small amounts and tamping with a rubber stopper on a glass rod each time before adding a new amount.

From the results given in the tables it is possible to note the effect of initial moisture content on moisture movement in a number of different ways.   With the exception of the Station silt loam, no matter how the comparison is made, it is seen that moisture movement was no greater in the soils of higher initial

TABLE 6

Results with sandy loam soil

INITIAL MOISTURE TREATMENT		OVEN-DRY	AIR-DRY TO OVEN-DRY	AIR-DRY DUPLICATE TUBES		HYGRO-SCOPIC COEFFICIENT	HYGRO-SCOPIC COEFFICIENT TO AIR-DRY	1¼ HYGRO-SCOPIC COEFFICIENT	1¼ HYGRO-SCOPIC COEFFICIENT TO AIR-DRY	2 HYGRO-SCOPIC COEFFICIENT	2 HYGROSCOPIC COEFFICIENT TO AIR-DRY DUPLICATE TUBES	
Distance of moisture movement in	20 minutes	5.8 cm.	5.6 cm.	5.3 cm.	5.9 cm.	5.0 cm.	5.5 cm.	5.5 cm.	5.9 cm.	3.6 cm.	5.3 cm.	5.7 cm.
	1¼ hours	11.9 cm.	12.0 cm.	11.6 cm.	11.9 cm.	11.2 cm.	11.1 cm.	11.1 cm.	11.5 cm.	7.8 cm.	10.7 cm.	11.0 cm.
	3 hours	15.7 cm.	16.0 cm.	15.8 cm.	16.1 cm.	15.3 cm.	15.0 cm.	14.5 cm.	15.2 cm.	10.6 cm.	13.8 cm.	14.3 cm.
	7¼ hours	22.0 cm.	22.6 cm.	23.0 cm.	22.9 cm.	22.5 cm.	21.4 cm.	20.6 cm.	21.5 cm.	15.5 cm.	19.2 cm.	19.6 cm.
	19 hours	29.0 cm.	29.9 cm.	31.3 cm.	31.1 cm.	30.8 cm.	28.5 cm.	27.2 cm.	28.2 cm.	20.5 cm.	24.9 cm.	25.2 cm.
Total water taken up		173 gm.	185 gm.	198 gm.	192 gm.	183 gm.	181 gm.	162 gm.	187 gm.	127 gm.	167 gm.	169 gm.
Initial moisture in soil		None	None	1.17%	1.17%	2.53%	0.81%	4.06%	0.82%	5.72%	0.99%	0.78%
Apparent specific gravity		1.485	1.468	1.464	1.459	1.445	1.440	1.376	1.374	1.331	1.323	1.316

TABLE 7

Results with Station silt loam soil

INITIAL MOISTURE TREATMENT		OVEN-DRY	AIR-DRY TO OVEN-DRY	AIR-DRY DUPLICATE TUBES		HYGROSCOPIC COEFFICIENT	HYGROSCOPIC COEFFICIENT TO AIR-DRY	1½ HYGROSCOPIC COEFFICIENT DUPLICATE TUBES		1½ HYGROSCOPIC COEFFICIENT TO AIR-DRY DUPLICATE TUBES	
Distance of moisture movement in	15 minutes	3.1 cm.	3.2 cm.	3.2 cm.	3.1 cm.	3.5 cm.	3.3 cm.	3.3 cm.	4.3 cm.	3.8 cm.	3.6 cm.
	1¾ hours	8.0 cm.	8.2 cm.	7.3 cm.	7.6 cm.	8.6 cm.	7.7 cm.	10.0 cm.	10.5 cm.	9.3 cm.	8.9 cm.
	2¾ hours	11.0 cm.	11.2 cm.	10.1 cm.	10.6 cm.	12.1 cm.	10.8 cm.	14.5 cm.	15.0 cm.	13.0 cm.	12.8 cm.
	17¾ hours	27.7 cm.	28.3 cm.	26.5 cm.	27.1 cm.	31.6 cm.	28.5 m.	35.0 cm.	35.0 cm.	31.7 cm.	31.0 cm.
Total water taken up		217 gm.	218 gm.	190 gm.	204 gm.	216 gm.	217 gm.	237 gm.	231 gm.	247 gm.	240 gm.
Initial moisture in soil		None	None	2.56%	2.56%	5.43%	2.67%	7.78%	7.78%	2.34%	2.45%
Apparent specific gravity		1.347	1.335	1.331	1.328	1.258	1.265	1.184	1.192	1.189	1.204

TABLE 8

Results with Purchase silt loam soil

INITIAL MOISTURE TREATMENT		OVEN-DRY	OVEN-DRY TO AIR-DRY BEFORE FILLING	AIR-DRY	HYGROSCOPIC COEFFICIENT	HYGROSCOPIC COEFFICIENT TO AIR-DRY	1½ HYGROSCOPIC COEFFICIENT	1½ HYGROSCOPIC COEFFICIENT TO AIR-DRY	2 HYGROSCOPIC COEFFICIENT	2 HYGROSCOPIC COEFFICIENT TO AIR-DRY
Distance of water movement	10 minutes	3.7 cm.	3.3 cm.	3.9 cm.	4.2 cm.	4.0 cm.	4.9 cm.	5.0 cm.	4.6 cm.	5.5 cm.
	30 minutes	8.5 cm.	7.1 cm.	8.1 cm.	8.7 cm.	8.5 cm.	9.9 cm.	10.4 cm.	9.9 cm.	11.1 cm.
	1¾ hours	13.4 cm.	11.0 cm.	12.7 cm.	13.8 cm.	13.4 cm.	15.8 cm.	16.0 cm.	15.6 cm.	17.2 cm.
	3 hours	17.9 cm.	14.8 cm.	17.0 cm.	18.5 cm.	18.1 cm.	21.0 cm.	21.2 cm.	20.5 cm.	22.5 cm.
	9 hours	29.3 cm.	23.5 cm.	27.6 cm.	30.5 cm.	29.2 cm.	32.7 cm.	33.4 cm.	31.2 cm.	34.5 cm.
Total water taken up		205 gm.	155 gm.	186 gm.	203 gm.	208 gm.	212 gm.	250 gm.	186 gm.	258 gm.
Initial moisture in soil		None	0.88%	1.43%	3.56%	1.10%	5.40%	1.27%	7.41%	1.33%
Apparent specific gravity		1.438	1.435	1.413	1.321	1.322	1.246	1.254	1.204	1.209

TABLE 9

Results with clay loam soil

INITIAL MOISTURE TREATMENT		OVEN-DRY	AIR-DRY TO OVEN-DRY	AIR-DRY DUPLICATE TUBES		HYGROSCOPIC COEFFICIENT	HYGROSCOPIC COEFFICIENT TO AIR-DRY	1½ HYGROSCOPIC COEFFICIENT DUPLICATE TUBES	1½ HYGROSCOPIC COEFFICIENT TO AIR-DRY DUPLICATE TUBES	1½ HYGROSCOPIC COEFFICIENT TO AIR-DRY DUPLICATE TUBES	2 HYGROSCOPIC COEFFICIENT TO AIR-DRY DUPLICATE TUBES
Distance of water movement	17 minutes	2.3 cm.	3.2 cm.	2.1 cm.	1.7 cm.	2.5 cm.	2.7 cm.	3.4 cm.	3.5 cm.	3.6 cm.	3.4 cm.
	2½ hours	7.0 cm.	8.0 cm.	6.3 cm.	6.0 cm.	7.9 cm.	7.7 cm.	9.2 cm.	9.5 cm.	9.5 cm.	9.2 cm.
	17¾ hours	16.6 cm.	18.5 cm.	16.0 cm.	15.8 cm.	20.2 cm.	19.9 cm.	21.8 cm.	21.8 cm.	22.4 cm.	22.1 cm.
	42¾ hours	23.9 cm.	26.9 cm.	23.5 cm.	23.5 cm.	29.1 cm.	28.2 cm.	29.9 cm.	29.8 cm.	30.0 cm.	29.7 cm.
	74 hours	30.0 cm.	33.8 cm.	29.4 cm.	29.4 cm.	34.9 cm.	33.9 cm.	34.9 cm.	35.0 cm.	34.8 cm.	34.7 cm.
Total water taken up		238 gm.	277 gm.	225 gm.	228 gm.	250 gm.	277 gm.	224 gm.	217 gm.	283 gm.	282 gm.
Initial moisture in soil		None	None	3.67%	3.67%	8.07%	3.24%	11.87%	11.87%	3.36%	3.54%
Apparent specific gravity		1.310	1.320	1.252	1.309	1.201	1.209	1.119	1.132	1.128	1.114

moisture content than in those of lower moisture content. The hygroscopic water and the presence of an additional amount of capillary water were both without apparent effect in this connection.

The Station silt loam gave no greater movement in the air-dry soil than in the oven-dry soil, but movement was appreciably greater in the soil of initial hygroscopic coefficient and $1\frac{1}{2}$ times the hygroscopic coefficient than in the air-dry soil. Results from preliminary experiments with this soil are also in substantial agreement with this in showing an increase in moisture movement due to the presence of initial amounts of moisture in addition to that in the air-dry soil.

On the whole, however, these results do not show any significant increase in capillary moisture movement due to the presence of small initial amounts of moisture in addition to either the moisture-free or the air-dry condition. Or, stated in another way, neither the air-dry nor the oven-dry soil condition offered any apparent resistance to movement of capillary water through soil as compared with movement in the same soil containing an additional small amount of initial moisture.

It may be called to mind that this has a practical bearing on the necessity of having loose earth mulches in a dry condition for their maximum functioning and also in the application to field conditions of data on the extent of capillary movement obtained by observations made on capillary movement of water in air-dry soils in the laboratory. Some experimental work very often referred to as showing greater capillary movement of water through soil in an initial moist condition than in the air-dry state is that of Briggs and Lapham with movement in dry and saturated sand (2). According to their results, the movement in saturated sand was operative through a distance 4.5 times that in the air-dry sand. It seemed rather reasonable, with this difference in movement in saturated and dry sand, that small differences in initial moisture content in field soils should show an appreciable effect. The fact that such was not found led to the repetition of this work with the dry and saturated sand.

The method of experimentation was practically that of Briggs and Laphan. Distance of movement in the air-dry sand was determined by filling glass tubes of 1-inch diameter with the air-dry sand and determining the maximum height of movement of capillary water from a vessel of water up into the sand; at the same time the maximum height of movement of water through the saturated sand was determined by filling a number of tubes of different lengths with the sand, saturating, and determining the maximum height through which water would be moved to the surface from a vessel of water in contact with the bottom. The fact that movement of water from the vessel to the surface of the sand was taking place could be told both by the sand's remaining moist at the surface and by the drop of water level in the reservoir vessel. The tubes used for determining the distance of movement in the saturated sand usually were of lengths increasing in 5-cm. steps. By remov-

ing the sand as it dried from the top of the tube found to be just above the height of maximum water movement until a permanently moist condition was reached, it was possible to locate fairly definitely within the 5-cm. interval the exact distance sought.

Quartz sand from three different sources was used in this work. (a) The first was sand secured from the Central Scientific Company; it was reasonably clean and in its natural condition gave very good moisture movement. It will be designated as C. S. sand. (b) Second, a sand was secured from the department of chemistry of the Experiment Station, which originally came from some point in Tennessee. This sand appeared clean, but in its natural state gave very poor capillary movement and on examination was found to contain a trace of oily material. It was never used without some preparatory cleaning treatment. This sand will be designated as Tenn. sand. Movement in the C. S. sand was also usually increased by a preparatory cleaning treatment. (c) Finally one experiment was made with a sand from the

TABLE 10

Movement in dry and saturated sand

SAND USED	MOVEMENT IN SAND		RATIO OF MOVE-MENT IN SATU-RATED TO MOVE-MENT IN AIR-DRY SAND
	Air-dry	Saturated	
	cm.	*cm.*	
Kentucky River, below 40-mesh................	25.5	33.0	1.30
C. S. below 60-mesh.........................	66.0	81.0	1.23
C. S. 40 to 60-mesh........................	33.0	58.0	1.77
C. S. 40 to 60-mesh.........................	25.0	45.0	1.80
C. S. 60 to 80-mesh..........................	37.3	60.0	1.61
Tenn. 60 to 80-mesh.........................	33.5	48.0	1.43
C. S. and Tenn. mixed, 80 to 100-mesh..........	41.3	75.0	1.81

sand deposits of the Kentucky River. The sand was used in its natural state. Both the C. S. and Tenn. sand were sieved and portions of different grades of fineness used in the work.

The results from this work are shown in table 10.

The variation between the different experiments makes impossible the assigning of any definite value to the ratio of movement of water in the sand in the two conditions. However, the variation is within reasonably small limits, and while the distance of movement is greater in the saturated than in the dry sand, yet the average ratio between the two of 1.56 shows a very much smaller increase in the saturated condition than that found by Briggs and Laphan

SUMMARY

Soils were placed in specially devised tubes and different initial moisture conditions brought about. These tubes were set in vertical positions with their lower ends in water, and the distance of penetration of water at various

times determined. It was found that small differences in initial moisture content of the soils were for the most part without significant effect in this connection, the distance of movement being as great in the oven-dry or air-dry soil as in soil containing an additional initial small amount of water.

A repetition of the work of Briggs and Laphan with movement in dry and saturated sand showed that movement in the saturated sand as an average of a number of experiments was 1.56 times that in the air-dry sand.

REFERENCES

(1) ALWAY, F. J., AND McDOLE, GUY R.   1917   Relation of movement of water in a soil to its hygroscopicity and initial moistness.   *In* Jour. Agr. Res., v. 10, p. 391–428.

(2) BRIGGS, LYMAN J., AND LAPHAM, MACY H.   1902   Capillary movement of water in dry and moist soils, U. S. Dept. Agr. Bur. Soils Bul. 19.

(3) HARRIS, F. S., AND TURPIN, H. W.   1917   Movement and distribution of moisture in the soil.   *In* Jour. Agr. Res., v. 10, p. 113–155.

(4) KARRAKER, P. E.   1915   Effect on soil moisture of changes in the surface tension of the soil solution brought about by the addition of soluble salts.   *In* Jour. Agr. Res., p. 4, p. 187–192.

ALUMINUM AS A FACTOR IN SOIL ACIDITY

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I. INTRODUCTION

In 1902, the late Dr. Cyril G. Hopkins (33) and his associates of the Illinois Experiment Station presented at the nineteenth annual meeting of the Association of Official Agricultural Chemists the first practical method for ascertaining quantitatively the acidity or lime requirements of a soil. The method is based on the theory that the acids, organic and mineral, in the soil are insoluble in water and cannot be extracted with water, but when a mineral salt solution is added to the acid soil, a double decomposition takes place, the acids (humic acids) react with the salt solution, uniting with the mineral base, forming neutral humates and liberating the mineral acid or an acid salt. The titration of the mineral acid serves as a basis for determining the total acidity of the soil. Eichhorn (16) found that soil rich in humus and containing free humic acids, liberates the acid from neutral salt solutions when brought in contact with such a soil. Solenow (64) also observed that the difficultly soluble organic acids of the humus liberate mineral acids which may affect the plants growing on the soil affected. These observations are at variance with that made by Knop and Detmer (69) who claim that generally "humates" and "humic acids" are much less soluble in salt solutions than in pure water. But Heiden and Schumacker (37) demonstrated that portions of salts in solution are removed by peat, prepared humic acids, and artificial humus when these come in contact with the salt solution. Veitch (80) is also of the opinion "that organic matter is able to remove from solution a portion of the mineral salt with which it is brought in contact," but further asserts that "none of the standard works on absorption of soils makes mention of the production of free mineral acids; neither theoretical considerations nor a cursory examination of the literature lead one to believe that mineral acids in amounts equivalent to the total organic acids are set free by the action of mineral salt solutions on acid organic material."

Shortly after the publication of the Hopkins method of soil acidity-determinations, Veitch (80) subjected it to a critical study. Tests were made for free hydrochloric acid in the extract, and except in one or two cases where the presence of water-soluble sulfuric acid was proven, there were no tests which showed a considerable amount of free acid. Tests for phosphoric acid gave a negative result. In a previous work on the solubility of soil ingredient

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in saline solutions, Veitch, however, noted the filtrates were frequently acid in reaction, and attributed it to the presence, in considerable quantities, of aluminum, iron and manganese in the solution. He further found that when the apparent acidity of the extract was equivalent to more than 1 or 2 cc. 0.05 N alkali a precipitate was formed on titration which he identified as the hydroxides of aluminum, iron and manganese. Ames and Schollenberger (3) by experiment undertook to demonstrate that free acid is formed by interchange, between acid soils and potassium nitrate solution. The procedure is described in Bulletin 306 of the Ohio Agricultural Experiment Station. The results obtained from 200 cc. of the potassium nitrate extracts are the following:

Acidity due to free acid, by titration with methyl orange..... 9.1 cc. 0.1 N NaOH
Total acidity to phenolphthalein........................ 39.9 cc. 0.1 N NaOH
Acidity due to salts of Fe and Al (by difference).......... 24.8 cc. 0.1 N NaOH
Chlorine equivalent to 0.3 cc. 0.1 N AgNO$_3$............... 0.0476 gm.
Silica, by loss after HF treatment....................... 0.0005 gm.
Ferric and aluminum oxides........................... 0.0471 gm.
Iron by reduction and titration as Fe$_2$O$_3$.................. 0.0136 gm.
Al$_2$O$_3$ by difference..................................... 0.0335 gm.
The iron was found equivalent to 5.0 cc. of 0.1 N NaOH; and the alumina to 19.7 cc.

It is thus seen that although free acid has developed the acidity due to the presence of aluminum is more than twice the acidity due to free acid. The following opinion by Veitch (80) fits well with this result as well as with his own: "It seems that there is no setting free of appreciable quantities of hydrochloric acid, and that there is practically no reaction between the organic matter and the salt solution, whereby difficultly soluble organic acids are dissolved, but that the acidity of the filtrate (or the acidity which is greater than would be given by water under the same condition) is due to the solution of alumina or some other acid-salt yielding base."

The author has also made some studies on the composition of potassium nitrate extracts of three acid soils. A more detailed account of the results of this study will be given in the latter part of this paper, but the conclusion he reached is that potassium nitrate solution brings into solution the aluminum in the acid soil when the latter is brought in contact with the salt solution; that the strong acidity exhibited by the potassium nitrate extract is due largely to the presence of a considerable amount of aluminum in solution; and that the precipitate formed at the point when the extract passed from an acid into an alkaline solution is largely aluminum hydroxide.

Hitherto, the significance of Veitch's discovery has never been appreciated, and the nature of soil acidity has generally been ascribed to the presence of free organic and mineral acids in the soil. The recent work, however, of such men as Abbott, Conner and Smalley (1) of Indiana, Ruprecht and Morse of Massachusetts (59) and Hartwell and Pember (27) of Rhode Island, in which they have shown the rôle aluminum plays in some acid soils, has given a new phase to the problem of soil acidity. The last two men, especially, are so

convinced of the presence of aluminum in acid soils as the cause of the differ-
ent behavior of barley and rye grown in an acid soil that they think "the
elimination of the effect of aluminum in acid soils seems likely to prove more
important than the neutralization of the acidity" and that "attention should
be given to methods of determining active aluminum while we are also devel-
oping those for soil acidity" (28). If aluminum should prove to be the most
important factor in acid soils, and a search is made for a method for deter-
mining active aluminum and for eliminating its effect in acid soils, such a
method already exists. Veitch had demonstrated that the Hopkins method
does not bring considerable free acids in an acid soil into solution but instead
brings the aluminum in the soil into solution; the application of the method
to field conditions has brought excellent results. In other words, the method
determines aluminum. The application of limestone to acid soils, according
to the method, eliminates the effect of aluminum, and so far as aluminum is
concerned in acid soils the Hopkins method is the best method for determining
and correcting soil acidity.

II. OPINIONS CONCERNING THE CAUSE AND NATURE OF SOIL ACIDITY

Frear (19) and Ames and Schollenberger (3) have already reviewed quite
comprehensively the literature and theories covering the subject of soil acidity
and in this paper the author only attempts to summarize briefly the opinions
expressed by the different investigators. These opinions or theories may be
divided into three groups.
  1. The presence of true acids.
  2. Adsorption by soils.
  3. The presence of considerable quantities of soluble aluminum salts.

1. The presence of true acids

In this group there are two important theories, the organic acid theory and
the mineral acid concept. Let us take up first the organic acid theory.

In the decomposition of plant and animal residues organic acids are pro-
duced. Under proper aeration these decomposition products are used up by
soil organisms as fast as they are formed. But in poorly aerated and drained
soils, these products may accumulate giving rise to acid condition. The acidity
of some peat and muck soils has long been known, and this acidity has been
assigned to complex insoluble organic acids. This theory was proposed by
Sprengel (65) after having discovered what he called humic acid. Berzelius
(6) further advanced the theory when, by treating a soil with an acid he ob-
tained two substances, one insoluble and the other soluble, the latter being
identical to the humic acid of Sprengel. Tacke and Süchting (71) held the
view that the acidity of humic acids can only be accounted for on the basis of
true acids. That organic acids exist in normal soils is a known fact. Blair
and Macy (8) in Florida found muck soils which gave an acid aqueous extract

after boiling the extract for a long time. They ascribed the acidity to soluble organic acids in the soil. The so-called humus has been subjected to a critical examination by Shorey (63) who found thus far at least thirteen organic acids, among which are oxalic acid, succinic acid, saccharic acid, acrylic acid, picoline carboxylic acid, paraffinic acid and lagnoceric acid. But it is believed that under ordinary conditions the organic substances present in the soil cannot bring a condition unfavorable to plant growth.

Some soils while deficient in organic matter are decidedly acid, hence it follows that the acidity in this case must be attributed to other causes. Truog (75) thought that the acidity in this case is caused by true acids. It is claimed that plants and certain hydrated compounds in the soil removed the bases from the salts leaving free acids. Stoddart (67) for example, is of the opinion that the sulfates and chlorides in the soil are split up, the base elements being absorbed by the plants, leaving the acid radicals as acids thus giving rise to an acid condition. It is further believed that the silicates which are important constituents of soils of non-limestone rock origin are decomposed by carbonated water in the soil resulting in the removal of bases which are either taken up by plants or leached out. The oxides of silicon and aluminum left over combine to form aluminum-silicic acids which may cause soil acidity. Truog (77) says: "It is possible that mere removal of bases from the original silicates may give rise to acid silicates which cause soil acidity." On this point Hopkins (32, p. 176) says, "Acid silicate is formed from polysilicates from which some basic elements may have been removed and replaced with acid hydrogen, by reaction with soluble organic acids, or possibly by the long-continued weak action of drainage waters charged with carbonic acid, do exist in the soil, and the evidence thus far secured indicates that they account for most of the acidity of soils which are at the same time strongly acid and very deficient in humus." In the study of acid red clay soils of Porto Rico, Loew (41) ascribes the acidity to the presence in the soils of an acid clay or aluminum silicates having the formula $H_4Al_2Si_2O_9$, which he called argillic acid.

Lastly by the use of electrometric and colorimetric methods of determining hydrogen-ion concentration, Gillespie (22) demonstrated the presence of acid in the soil. His findings were in accordance with the results of Sharp and Hoagland (62), who concluded that soil acidity is due to the presence of an excess of hydrogen ions in the soil solution.

It is believed that certain treatments of the soil may also give rise to acidity in the soil. Continued application, for example, of artificial fertilizers like sulfate of ammonia and acid phosphate causes acidity of the soil. When sulfate of ammonia is applied to soils, ammonia is nitrified leaving the sulfate radical to form sulfuric acid. Muriate of potash, according to Stoddart (67) tends to leave an acid residue due to the absorption of potassium by plants or soil colloids, leaving free sulfuric and hydrochloric acids. There are instances in which continued application of ammonium sulfate to the soil resulted in an acid soil. Wheeler (82) reported acid the soil in the plots of the Rhode

Island Experiment Station which received ammonium sulfate continuously. Hall and Gimingham (24) in England, Hunt (35) in Pennsylvania and Ruprecht and Morse (60) in Massachusetts encountered similar results with experimental plots receiving ammonium sulfate continuously but not limed.

It has also been thought that acid phosphate may produce acidity in the soil. The fact that acid phosphate is an acid salt is responsible for this belief. In discussing the advantages of raw phosphate over acidulated phosphate Hopkins (32, p. 242) says: "A third point in favor of raw phosphate in common with bonemeal and slag, is that it is free from acidity and has no tendency to injure the soil. This is a minor advantage, because if acidity develops from the use of acid phosphate (and it does) it can be corrected at a small expense by the addition of any form of lime." Thorne (72) of the Ohio Experiment Station also is of the opinion that acid phosphate may develop acidity in the soil. He says: "There is reason to believe that acid phosphate increases the tendency to soil acidity, but it is not the sole cause of such acidity for there are very acid soils which have never received any phosphate." These opinions, however, do not agree with the experimental evidence. In a study of the acidity of experimental plots in Indiana in which acid phosphate has been applied for twenty years, Conner (13) found that these plots show less acidity than soils which have never had acid phosphate. By computing the amount of free phosphoric acid added to the soil when the rate of application is 200 pounds of acid phosphate containing 14 per cent available phosphoric acid, Frear (19) concluded that it would take a long time and a large amount of phosphate to make a soil acid by such direct action. The results of the author's tests which will be presented in this paper also indicate that acid phosphate tends to reduce rather than increase the acidity of the soil.

2. The adsorption theory of soil acidity

The phenomenon of soil acidity has also been explained as a case of adsorption. Cameron (11) was the first to apply the theory of adsorption in explaining the acid reaction of certain soils. He attacked the blue litmus paper test for soil acidity on the ground that wet cotton also turns blue litmus paper red. He is of the opinion that the reddening of blue litmus paper by certain soils is a case of selective adsorption.

After investigating some acid soils of Michigan, Harris (25, 26) arrived at the conclusion that the acid reaction of the soil is due to selective adsorption and not to the presence of acids.

Parker (49) asserts that because of the nature of the surface of its constituents soils adsorb the cation at a greater rate than the anion, and that the acid reaction of certain soils is due to this fact.

In a comprehensive study of acid soils of Japan, Daikuhara (14) concludes that their acid reaction is not due to organic acids (humus) alone but also to adsorption of colloidal compounds of aluminum and iron.

Gully (23) also ascribes the acid reaction of peat moss and peat soils to adsorption of the colloidal matter of the covering of the sphagnum cells.

3. *The presence of soluble salts of aluminum in the soil*

The idea had its inception in the work of Abbott, Conner, and Smaller (1) who investigated a few years ago, the causes of the unproductivity of some soils in Indiana. They obtained water extract of the acids soils, and determined its composition. They have found that the extract reacts acid to phenolphthalein and that the nitrate was present partly as aluminum nitrate. Corn seedlings were grown in the extract along side of solutions of nitric acid and aluminum nitrate of known normality, and it was found that the extract was extremely toxic up to $0.0005 N$. It was found that the toxicity of the extract was equal to the toxicity of nitric acid and aluminum nitrate of the same normality; and the conclusion arrived at was that soluble salts of aluminum are largely responsible for the unproductiveness of the soils in question.

Ruprecht and Morse (60), investigating the effect of continued application of ammonium sulfate to soils, found that aluminum sulfate is formed which causes the acid reaction and the unproductivity of the soil.

Hartwell and Pember (27) carried on a comprehensive search for the cause of the different behaviour of rye and barley grown on soils from plots continuously receiving ammonium sulfate. Different inorganic substances have been subjected to experiment to discover the most active factor and the conclusion reached was that aluminum is the element responsible for the depression of the growth of barley.

After reviewing the different theories concerning the nature and cause of soil acidity Ames and Schollenberger (3) expressed the following opinion: "The theory of the existence of silicic or alumina-silicic acids in the soil would serve as a complete explanation for all the observed phenomena; the conception is simple and is supported by analogy with bettter known reactions which is as much as can be said for any of the theories which have been offered."

The work of Abbott, Conner and Smalley, Ruprecht and Morse, and of Hartwell and Pember, however, has opened up new possibilities by which the nature and causes of soil acidity could be studied further. With the hope that more light might be thrown upon aluminum as a factor in soil acidity the present work has been undertaken, bearing in mind three facts. First, aluminum salts are highly toxic at a certain concentration; second, aluminum is abundant in the soil, being next to oxygen and silicon; and third, plants absorb bases and calcium carbonate is leached out of the soil resulting in the depletion of the soil of this compound and enabling the aluminum in the soil to act as a base.

III. ALUMINUM IN AGRICULTURE

Aluminum is universally known as a non-essential element to plants. Hydrated silicates and oxides of aluminum, however, are believed to exercise great influence in holding some of the plant-food elements in the soil, preventing their loss in drainage water. Aside from this, aluminum has no economic

value in agriculture. As stimulants or as fertilizers very little is known of aluminum compounds and the few scattered experiments on this subject are incomplete and inconclusive. On the other hand, aluminum has been found extremely toxic to plants. Since aluminum is abundant in the soil and under certain conditions becomes harmful to plants, as in the case of some acid soils, it is in this fact that aluminum will prove of great importance to the agriculturist.

Aluminum in plants

Although aluminum is not an essential element, analyses of ashes show that it is taken up by plants. It is, however, present in small amounts in most plants. Pfeffer (50) speaks of the abundance of aluminum in *Lycopodium chamaecyparissus* and *L. alpinum*, where it constitutes from 22 to 27 per cent of the ash, while in certain species of *Lycopodium* only traces are found. Johnson (37) states that aluminum is found in small amounts in the ashes of agricultural plants, but added that it is not clear whether it is an ingredient of the plants or due to particles of clay adhering to plants. Robinson, Steinkoenig and Miller (57) report analyses of legumes, vetetables, grasses, trees, shrubs, and show that aluminum is found in all the plants analyzed. The form in which aluminum is present in the plant is not known. According to Berzelius, aluminum as alumina is united with tartaric acid, and according the Ritthausen with malic acid [quoted by Johnson (37)]. Pfeffer (50), however, is not certain whether aluminum in *Lycopodium* is present in the form of tartrate. In a study of the aluminum contents of certain vegetables including corn and corn products, hominy, oatmeal, carrots and white and sweet potatoes, Meyers (44) found that aluminum in these vegetables is found in a soluble form, and averred that a relatively large consumption of aluminum may result from a diet consisting chiefly of vegetables.

Physiological action of aluminum on plants. According to Jost (38) Jamano found aluminum to be of service in the development of barley. This is in conflict with the results of Hartwell and Pember (27), in which they show that the depression of the growth of barley in an acid soil is due to the presence of aluminum. Maze (43) also asserts that aluminum is necessary for the best growth of maize. Experimental evidences, however, point to the fact that aluminum is not only a non-essential element but it is also very harmful to plants under certain conditions.

Fluri (18) describes certain experiments carried out on *Spirogyra*, *Elodea*, and *Lemara* with sulfate, nitrate, chlorate and bichromate of aluminum. He found that in light, production of starch is reduced, but also found that while assimilation was checked it was not inhibited. The aluminum found in the cell was small and the action could not then be attributed to a chemical reaction. But as starch production was affected it was thought that the action of aluminum was on the diastase.

Hebert (29) made some germination tests of peas, wheat and rape with sulfates of aluminum and other metals and found that the salts were strongly

poisonous. Varvaro (79) also reports that aluminum oxide, like the oxides of manganese, iron, uranium, cerium, copper, zinc, cadmium, mercury and lead has a retarding effect on the germination of kidney beans, but has an accelerating effect in the case of corn. Experimenting on the effect of different aluminum salts on the germination of wheat, Micheels and DeHeen (45) found that while kaloin and alumina were somewhat beneficial, the salts were very harmful.

In investigating the effect of different salts of aluminum on the growth of *Zea mays, Vicia faba, Leus esculenta* and *Helianthus annuus* Kratzmann (39) found that the growth was hindered by the salts when the concentration was 0.005 per cent, but stimulated when the concentration was only 0.0001 per cent. *Aluminum nitrate showed a toxic effect. In this connection, significant is the statement of House and Gies (35)* that the toxicity of aluminum salts depends upon the concentration of the solution. Yamano (84) found that moderate amounts of aluminum salts have a stimulating effect upon the development of barley and flax. He further found that in water culture 0.2 per cent of alum proved injurious after three weeks while 0.8 per cent killed the plant in a few days. Miyake (46) also found that the aluminum chloride is toxic even in dilute solution. The toxicity appeared when the concentration was greater than 0.000133. It was further found that the toxicity of aluminum chloride was approximately equal to that of hydrochloric acid of the same normality. Under the supervision of Professor C. F. Hottes of the Department of Plant Physiology, the author carried on some experiments on the toxicity of aluminum sulfate to barley. Solutions of 0.01 N, 0.001 N, 0.0001 N, and 0.00005 N were prepared, and barley seedlings were grown in them. The author found that the average growth of 10 plants for 7 days was 70.5 mm. in the control; 45.5 mm. in 0.01 N; 65 mm. in 0.001 N; 71.5 mm. in 0.0001 N and 78.9 mm. in 0.00005 N. It is thus seen that 0.01 N is highly toxic; 0.001 N depresses growth; and 0.0001 N has no effect at all. In 0.00005 N stimulating effect was noted. It was further observed that the seedlings growing the first two dilutions had root systems more than three times as greatly depressed as those growing in the control or in any of the two weaker solutions. The limit of toxicity lies probably between 0.0001 N and 0.001 N.

Other investigators who have proved the toxicity of aluminum salts are Abbott, Conner and Smalley; Ruprecht and Morse; and Hartwell and Pember, whose works have been already mentioned in the preceding discussion. It may be said, however, that it was not until the work of these men appeared that the toxicity of aluminum salts has been linked with the soil as a contributing factor in soil acidity.

Aluminum salts as stimulants and fertilizers

Experiments have been made to ascertain the value of aluminum salts as catalyzers or stimulants. Pfeiffer and Blanck (51) found that small amounts of aluminum sulfate combined with a small portion of manganese sulfate

caused an increase in the yield of dry substance in the grain, but an increase of the salt reduced the yield. Stoklasa (68) reports results from experiments on catalytic fertilizers for sugar beets. He showed that a combined application of 9 kgm. (19.8 lbs.) of manganese and 4.48 kgm. (9.8 lbs.) of aluminum sulfate per hectare has increased the yield of sugar beets from 30 to 50 per cent. He is of the opinion that aluminum, like manganese, zinc, and copper, is a catalytic agent, performing a function in the assimilation of carbon by promoting rapid photosynthesis. Boullanger (9) made a comparative study of the catalytic value of the sulfates of aluminum, manganese, ferrous iron and uranium, and found that while the results obtained were not uniform, in the majority of cases they increased the yield. In the case of aluminum nitrate, however, the experience of Munerate, Mezzadroli and Zapparole (47) was different. They carried on a comparative test of the stimulating value of aluminum nitrate and sulfate together with the sulfate, chloride, dioxide and carbonate of manganese, boric acid, borate of soda and sulfate of uranium. The results showed that the lowest yield of sugar beets was obtained in the plot which received 100 kgm. (220 lbs.) per hectare of aluminum nitrate.

An attempt has been made also to find the effect on the productivity of the soil by the application of aluminum silicates. Voelcker (81) reports pot culture experiments in which green manures were associated with aluminum silicates, sodium silicates, kaolin, lime and magnesia. The results obtained showed that kaolin did not increase the yield of crops, but aluminum silicates with mustard as a green manure caused a large increase in the crop.

Finally, experiments have been carried out to determine the value of insoluble aluminum phosphate as a source of phosphorus to the plants. Prianishnikov (52) describes sand culture experiments in which wheat, oats, barley, peas and buckwheat were fertilized with aluminum phosphate alone and with calcium carbonate. The conclusion reached was that aluminum phosphate is assimilated, and that calcium carbonate had no appreciable depressing effect on the assimilability of aluminum phosphate. Baguley (4) reports a comparative test of orthophosphates of iron, calcium and aluminum on oats, peas and Swedish turnips grown on sand and chalk. The results obtained were better with iron and aluminum phosphates than with calcium phosphate. Truog (74) also presents results of experiments carried out in the greenhouse with ten different kinds of plants manured with rock phosphate, precipitated calcium phosphate, and phosphate of aluminum, iron and manganese. The results obtained were summarized as follows: "Contrary to the general belief that aluminum and iron phosphates are relatively unavailable to plants, nine of the ten plants tested made better growth on aluminum phosphate than on calcium phosphate, and six better growth on iron (ferric) phosphate." In another publication (76) in which results from a more comprehensive series of experiments on phosphate involving a large number of plants, were presented, he draws this conclusion: "Precipitated ferric and aluminum phosphates produced with a few exceptions good growth and in a few cases even better growth than the acid phosphate."

Aluminum in the soil

Aluminum is the most widely scattered metal (53) and next to oxygen and silicon is the most abundant element. It constitutes 7.85 per cent of the lithosphere and 7.30 per cent of the lithosphere and atmosphere combined (12). It does not occur in nature in the free state, but in combination with oxygen, the alkalies, flourine, silicon, the acids, etc., it forms minerals and rocks which on disintegration become the bases of soils and clays. Aluminum is present in the soil as the oxide, hydroxide, hydrated oxides, phosphate and silicates (64). In order to give some idea as to the amount of aluminum present in the soil, analyses of some soils in America are given in table 1.

TABLE 2

Chemical analyses of some American soils

CONSTITUENTS	ADOBE SOILS		COASTAL PLAINS PROVINCE	LIME-STONE VALLEY AND UPLAND PROVINCE	PIED-MONT PLATEAU PROVINCE	GREAT PLAINS PROVINCE	GLACIAL AND LOESSIAL PROVINCE	RIVER FLOOD PLAINS PROVINCE
	A	B	No. 1	No. 3	No. 15	(1)	(5)	(13)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
SiO_2	19.24	66.69	94.50	79.25	66.49	78.85	76.81	93.29
Al_2O_3	3.26	14.16	2.07	8.89	17.11	9.68	9.73	2.45
Fe_2O_3	1.09	4.38	0.83	4.44	7.43	2.72	3.26	0.78
MnO	Trace	0.09	0.007	0.07	0.51	0.036	0.068	0.066
MgO	2.75	1.28	0.09	0.33	0.31	0.72	0.60	0.01
CaO	38.94	2.49	0.39	0.63	0.36	0.94	0.92	0.15
Na_2O	Trace	0.67	0.11	0.24	0.16	2.02	1.74	0.03
K_2O	Trace	1.21	0.10	0.67	0.62	2.31	2.20	0.45
H_2O	1.67	4.94						
P_2O_5	0.23	0.29	0.06	0.18	0.17	0.11	0.12	0.06
CO_2	29.57	0.77						
Organic matter	2.96	2.00	1.13	1.96	1.26			
SO_3	0.53	0.41	0.07	0.13	0.07	0.07	0.11	0.10
Cl	0.11	0.34						
Loss on ignition			1.74	4.80	8.06	2.28	4.09	2.12

The first two columns are adopted from Clarke (12), the next three from Robinson (55) and the last three from Robinson, Steinkoenig and Fry (56). The soil indicated A, is from Salt Lake City, Utah; B is from Santa Fe, New Mexico; No. 1 is Norfolk sandy loam, 3 miles southwest of Laurinburg, North Carolina, depth 0 to 14 inches; No. 2 is Decatur clay loam, 1 mile east of Hollywood, Alabama, depth 0 to 4 inches; No. 15 is Cecil clay 2½ miles northwest of Charlotte, North Carolina, depth 0 to 6 inches; (1) is Colorado sand near Greeley, Colorado, depth 0 to 14 inches; (5) is Knox silt loam, 2 miles north of Farley, Missouri, depth 0 to 14 inches; and (13) is Cahaba very fine sandy loam, Minden, Louisiana, depth 0 to 12 inches. For further details about these soils the reader is referred to the publications of these men.

By recalculation the total aluminum in these soils per acre of 2,000,000 pounds of surface soil amounts to 34,576 pounds for Salt Lake City adobe; 151,181 pounds for Santa Fe adobe; 21,954 pounds for Norfolk sandy loam, North Carolina; 94,237 pounds for Decatur clay loam, Alabama; 181,469 pounds for Cecil clay, North Carolina; 102,666 pounds for Colorado sand; 103,196 pounds for Knox silt loam, Missouri; and 25,985 pounds for Cahaba very fine sandy loam, Louisiana.

Burd (10) also reports total analyses of certain silty clay loam and fine sandy loam soils in California in which, for example, one silty loam soil and one fine sandy loam soil contain 14.03 per cent and 16.73 per cent alumina, or 148,802 pounds and 177,438 pounds aluminum per acre, respectively.

Aluminum in the subsoil. Analyses of the subsoils of soils given in the preceding table show larger quantities of aluminum. For example, the subsoil of Decatur clay loam contains 3 per cent more alumina than the surface soil. In every one of the ten subsoils analyzed by Robinson (55) alumina is higher than in the surface.

TABLE 2

Alumina in soil separates

SEPARATES	HEAVY LOAM	LOAMY LOESS SOIL	COARSE SANDY GNEISS SOIL
	per cent	*per cent*	*per cent*
Coarse dust, 0.25 to 0.01 mm	1.63	7.28	18.71
Medium dust, 0.01 to 0.005 mm	15.20	14.20	24.20
Fine dust, 0.005 to 0.0015 mm	20.48	19.41	30.21
"Clay" (Schlamm), 0.0015 to 0 mm	27.76	29.97	32.42

Distribution of aluminum in the soil separates. There have been a few attempts to determine the distribution of the chemical constituents of the soil in the different soil separates. Puchner, quoted by Failyer, Smith and Wade (17), presents data of chemical analyses for separates of three types of soil. The percentage of alumina found is given in table 2.

Steinkoenig (66) also reports determination of certain constituents of separates of ten loam soils from New York, North Carolina, Pennsylvania, South Carolina, Virginia, New Hampshire and Wisconsin. The average alumina found in the separates of these soils, together with the maximum and minimum is given below:

	FINE SAND	COARSE SILT	FINE SILT AND FINE CLAY
	per cent	*per cent*	*per cent*
Average	5.48	8.44	22.57
Maximum	12.56	18.28	31.33
Minimum	0.40	1.48	16.76

From the data above it can be seen that the largest quantity of aluminum is found in the finest particles of the soil and that the quantity diminishes as the particles become coarser. It follows from this fact that the more clayey the soil is the higher is the aluminum content, and this seems to be the case if the Cecil clay is taken as proof.

The aluminum compound in the soil that gives rise to soluble aluminum salts. Mention has been made before that aluminum is present in the soil as oxide, hydrated oxides, hydroxides, phosphates and silicates. But which of these compounds breaks up so readily in the soil to form soluble salts that proved injurious to crops in some soils, as has been found by Abbott, Conner and Smalley, and Ruprecht and Morse? We naturally look upon hydroxides. There are three forms of aluminum hydroxides recognized: Diaspore—$Al_2O_3 \cdot H_2O$, bauxite—$Al_2O_3 \cdot 2H_2O$, and gibbsite, otherwise called hydrar-gillite or oxyhydrates, $Al_2O_3 \cdot 3H_2O$ (53). Do these three forms behave chemically the same with mineral acids? Diaspore and bauxite are insoluble in cold and hot water and in acids and alkalies, but gibbsite, while not soluble in cold and hot water, is soluble in acid and alkalies (48). Moreover, the so-called aluminum salts, aluminum nitrate, $Al_2O_6(NO_2)_6$, aluminum acetate, $Al_2O_6(C_2H_3O)_6$, aluminum sulfates, $Al_2O_6(SO_2)_3$, and aluminum phosphate $Al_2O_6(PO_4)_2$—are chemically considered as derivatives of the oxyhydrates (53). Gibbsite, therefore, answers the first question, and the next question that comes up is, whether gibbsite is present in the soils of America.

Lateritization in northern climates. In the decomposition of rocks an insoluble residue made up mainly of silica, alumina and ferric oxide, and combined with water, is left over. When kaolinite is the predominant constituent of the residue it is called clay, but when hydrates of alumina and iron predominate the residue is called laterite. Hence, the process of rock decomposition in which kaolinite is the end product is called kaolinization and that process in which hydrates of alumina and limonite are the ultimate products is called lateritization (15). In regard to the latter process Clarke (12) says, "In the tropical and subtropical regions the processes of rock decay are often carried further than is usually the case within the temperate zones. The leaching is more complete, the silicates are more thoroughly decomposed, and the residues are richer in hydroxides."

There is a general opinion among geologists that kaolinization is characteristic of rock decomposition in northern climates while lateritization is characteristic of that in tropical and subtropical regions. For this reason there is a diversity of opinion as to the occurrence of aluminum hydrates in the soils of America. Cameron and Bell (11) for example, state that "either gibbsite or bauxite is but seldom found in soil," and that in the examination of several thousand soils from all over the United States, in only one soil, that which comes from southern California, was aluminum hydroxide observed. Lindgren (40) is also of the opinion that little or no aluminum hydroxide is formed in ordinary rock weathering, and that the occurrence of bauxite is a rarity in

the temperate climate.   On the other hand, Edwards (15) by recalculating analytical data for clays from different states, shows that bauxite exists in 21 states besides those in which the mineral exists in deposits of commercial value. In regard to the independence of bauxite as a mineral species Lindgren (40) says, "The independence of bauxite as a mineral species, is, however, questioned and it is believed rather to be a mixture of diaspore and gibbsite.   The Georgia bauxite according to T. L. Watson corresponds well to gibbsite." Hilgard (30) found a high ratio of alumina to soluble silica in some of the soils he examined, and could not attribute it to other than the presence of hydrous alumina, possibly gibbsite.   Beyer and Williams (7) reporting the analyses of flint fire clays from Missouri and New Jersey, also found a higher ratio of alumina to silica than that found in kaolinite but attributed it to the presence of a more highly aluminous silicate which he called pholerite.   In the reported analyses of ball and flint clays from Missouri and of fire clays from Pennsylvania, Rolfe (58), again, found a high proportion of alumina to silica, and attributed this to the presence in the clays of gibbsite or other minerals high in alumina. Ries (54) believes that there is the possibility that in kaolins high in aluminum bauxite or gibbsite might be present.   Finally, Galpin (21) in the study of flint clays and their associates encountered highly aluminous fire clays from near St. Louis, Missouri, and proves that the excess of alumina to silica is due to the presence of gibbsite.

The author does not pretend to show that aluminum hydrates are of common occurrence in the soils in America, but with the evidence gathered from the works of the men mentioned above he can not help reaching the conclusion that in some soils in the United States hydrates of alumina are present, and that in the case of soils where sufficient amounts of soluble salts of aluminum are found to be harmful to crops, the aluminum compound furnishing the aluminum is gibbsite, and until further investigations prove the contrary the author will hold to this view.

IV. EXPERIMENTAL

The problem

The work reported in this paper has been undertaken with the view of gaining some information on the following questions:

1. Aluminum is found in the soil in abundance and in conditions of varying stability.   When an acid soil is extracted with potassium nitrate solution aluminum is brought into solution and is largely responsible for the acid reaction of the extract.   Is not the acidity of the so-called acid soil due to the presence of active aluminum in the soil?

2. Sweet clover does not grow on a strongly acid soil while other plants have their growth depressed.   Since aluminum salts have been found highly toxic even in dilute solutions, is not this behavior a reaction to the toxicity of soluble salts of aluminum in the soil?

3. When acid soils are treated with limestone according to the potassium nitrate method, sweet clover thrives well. Does not calcium carbonate eliminate the toxicity of aluminum? And if so, how does it act?

4. Does acid phosphate increase the acidity of an acid soil?

5. If the acidity of the soil is due to the presence of active aluminum, what effects have soluble salts of aluminum on sweet clover grown in sand? What effect has aluminum salts on sweet clover in the presence of calcium carbonate, or acid phosphate?

General plan of the work

Based on the foregoing propositions, plans have been carried out:

1. To study the potassium nitrate extract of an acid soil before and after the application of limestone.

2. (a) To leach out a considerable quantity of acid soils with potassium nitrate and with water until the last 125 cc. of leachings no longer indicate acidity, and to grow crops on it.

(b) To analyze the leached out soils for aluminum, iron and manganese.

3. (a) To grow crops on acid soils, treated with limestone and acid phosphate, alone and in combination with each other, and in different amounts.

(b) To set aside a similar series as above, giving the same treatment except the growing of crops, for acidity determinations in two different periods.

4. To grow crops on sand treated with aluminum sulfate, aluminum chloride and aluminum nitrate and aluminum hydroxide, alone and in combination with calcium carbonate or with acid phosphate.

Description of the material used

Three types of soil have been secured from southern Illinois for this work. They are—gray silt loam, on tight clay of the lower Illinoisan glaciation area; yellow gray silt loam, an upland timber soil; and yellow silt loam from the unglaciated areas. All the soils were acid to the blue litmus paper test. The physical composition of these soils is given in table 3. The Bureau of Soil's method and grades of mechanical separation have been adopted in this analysis (42). Some of the chemical constituents of the soils are given also in column 2, table 4. Except for aluminum, iron and manganese, the methods of chemical analysis used were those of the University of Illinois Agricultural Experiment Station. Aluminum, iron and manganese were determined by a combination of some of Hillebrand's procedures and of some in Treadwell's "Qualitative Analyses." The sample was fused with sodium bicarbonate, and the subsequent steps as directed in Hillebrand's methods, were followed up to the point of precipitating aluminum and iron. The ammonium persulfate method was adopted at this point to precipitate the manganese together with aluminum and iron (31). Manganese was then separated from aluminum and iron by the barium carbonate method and determined as manganese

pyrophosphate as directed in Treadwell's process (73). Aluminum was separated from iron by the potassium hydroxide procedure and both were weighed as oxides, also according to the direction of Treadwell.

Gray silt loam. This is a surface soil taken from the border of one of the control plots of the experimental fields at Newton, Jasper County. It contains 98.24 per cent dry matter. The reaction as tested in the laboratory is acid, and the acidity or lime requirements according to the Hopkins method is 2125 pounds of limestone of 93 per cent purity, per acre (2,000,000 pounds of soil 6⅔ inches). The amounts of essential plant-food elements found are: nitrate-nitrogen 26 pounds per acre; total nitrogen 2900 pounds; phosphorus 1104 pounds; potassium 25,130 pounds; calcium 4510 pounds; magnesium 4520 pounds; and iron 47,800 pounds. Besides these the soil contains 840 pounds of manganese and 121,000 pounds of aluminum per acre.

. TABLE 3

Physical analysis of the soil (grades of Bureau of Soils)

CONSTITUENTS	SIZE OF PARTICLES	GRAY SILT	YELLOW GRAY SILT	YELLOW SILT
	mm.	*per cent*	*per cent*	*per cent*
Moisture......................		1.76	1.36	1.61
Fine gravel.....................	2–1*	0.93	1.37	0.00
Coarse sand....................	1.0 –0.5	2.15	1.79	0.21
Medium sand..................	0.5 –0.25	5.77	2.57	0.44
Fine sand......................	0.25–0.1	10.93	4.09	0.97
Very fine sand.................	0.1 –0.05	25.26	20.72	52.35
Silt...........................	0.05–0.005	44.61	50.99	19.54
Clay (by difference).............	0.005	10.15	18.45	26.47
Total.......................		99 80	99.99	99.98

* Calculated on water-free basis.

Yellow gray silt loam. This was taken from the farm of Joseph Quiztell at Carmi, White County, Illinois. It contains 98.64 per cent of dry matter. The reaction is acid and the lime requirements amount to 2814 pounds of limestone per acre. The essential plant-food elements found amount to 36 pounds of nitrate nitrogen per acre; 1370 pounds of total nitrogen; 693 pounds of phosphorus; 35,800 pounds of potassium; 3920 pounds of calcium; 4180 pounds of magnesium; and 74,200 pounds of iron. The manganese and aluminum found amount to 786 pounds and 151,000 pounds, respectively.

Yellow silt loam. This was taken near Vienna, Johnson County. It contains 98.39 per cent of dry matter. The reaction is acid and the lime requirement amounts to 2921 pounds per acre. The essential plant-food elements run up to 60 pounds of nitrate-nitrogen per acre; 1966 pounds of total nitrogen; 691 pounds of phosphorus; 29,000 pounds of potassium; 7850 pounds of calcium; 5330 pounds of magnesium and 74,200 pounds of iron. Manganese and aluminum reached 660 pounds and 14,900 pounds per acre, respectively.

TABLE 4
Gray silt loam

DETERMINED	ORIGINAL		EXTRACTED WITH KNO₃		EXTRACTED WITH H₂O	
Dry matter	98.24 per cent		98.15 per cent		98.28 per cent	
	P.p.m.[1]	Pounds per acre[2]	P.p.m.	Per cent extracted	P.p.m.	Per cent extracted
Acidity	988	2,125	30	96.96	925	7.31
Aluminum	60,500	121,000	33,400	44.79	49,800	17.67
Calcium	2,255	4,510	2,225	1.53	2,253	0.08
Iron	23,900	47,800	18,300	23.85	22,100	7:53
Magnesium	2,260	4,520	2,250	0.43	2.259	—[4]
Manganese	420	840	400	4.76	415	1.19
Nitrate-nitrogen	13	26	29	22.69[3]	11	15.39
Nitrogen	1,450	2,900	1,451	—[4]	1,450	—[4]
Phosphorus	550	1,104	481	12.54	549	—[4]
Potassium	12,560	25,130	40,140	28.95	12,420	1.11

Yellow gray silt loam

DETERMINED	ORIGINAL		EXTRACTED WITH KNO₃		EXTRACTED WITH H₂O	
Dry matter	96.64 per cent		99.03 per cent		98.93 per cent	
	P.p.m.	Pounds per acre	P.p.m.	Per cent extracted	P.p.m.	Per cent extracted
Acidity	1,358	2,813	11	99.93	1,260	7.21
Aluminum	75,600	151,200	30,300	59.93	58,900	24.73
Calcium	1,960	3,920	1,950	0.51	1,958	—[4]
Iron	20,100	40,300	16,700	14.44	19,600	2.48
Magnesium	2,095	4,180	2,090	—[4]	2,090	—[4]
Manganese	393	786	391	3.03	389	1.02
Nitrate-nitrogen	18	36	40	55.00[3]	15	16.66
Nitrogen	685	1,370	690	—[4]	689	—[4]
Phosphorus	336	693	275	18.15[3]	335	—[4]
Potassium	17,900	35,800	24,100	25.72	17,880	0.11

Yellow silt loam

DETERMINED	ORIGINAL		EXTRACTED WITH KNO₃		EXTRACTED WITH H₂O	
Dry matter	98.39 per cent		98.84 per cent		98.39 per cent	
	P.p.m.	Pounds per acre	P.p.m.	Per cent extracted	P.p.m.	Per cent extracted
Acidity	1,318	2,921	28	97.93	1,155	13.36
Aluminum	74,700	149,400	36,900	50.61	58,600	21.55
Calcium	3,425	7,850	3,400	0.72	3,410	0.43
Iron	37,100	74,200	2,930	21.01	33,900	8.62
Magnesium	2,665	5,330	2,660	—[4]	2,664	—[4]
Manganese	330	660	301	8.79	325	1.51
Nitrate-nitrogen	30	60	315	90.47[3]	23	23.33
Nitrogen	983	1,966	983	—[4]	981	—[4]
Phosphorus	346	691	292	15.61	339	2.02
Potassium	14,500	29,000	22,300	28.57[3]	14,400	0.69

[1] CaCO₃.

[2] Limestone requirements 2,000,000 pounds of soil.

[3] Increase.

[4] Within the limits of probable error.

While a considerable amount of a calcium is present in these soils, qualitative tests for carbonates showed only traces, which indicate that these soils are deficient in calcium carbonate. It may be added further that while these soils are well provided with potassium, the phosphorus and nitrogen contents are rather low. On the other hand, the aluminum content is very high.

Sweet clover (biennial variety) was the crop used in this work for the reason that it does not thrive in strongly acid soils, and will, therefore, respond more readily to soil treatment. Inoculated seeds were used in every case.

The experiments were carried out in 1-gallon pots, each holding about 5 kgm. (11 pounds) of soil.

Experiment I. Effect of aluminum salts, alone and in combination with calcium carbonate or with acid phosphate on sweet clover grown in sand

Three salts, aluminum sulfate, aluminum chloride and aluminum nitrate, and one hydroxide, aluminum monohydroxide, were used. When applied alone the chemicals were used in three different amounts, one, for the sake of convenience, is called normal application, the second, one-fifth the normal, and the third, five times the normal application. The basis for the normal application is the acidity or lime requirement of the yellow silt loam, which is 2921 pounds limestone per acre, or 6.79 gm. calcium carbonate per 5 kgm. of soil. In other words, the normal application is the chemical equivalent of the salts to 6.79 gm. of calcium carbonate. In combination with calcium carbonate or with acid phosphate the salts remained constant while calcium carbonate and acid phosphate were applied in three different amounts, normal, one-fifth normal and five times normal. The normal application of calcium carbonate is the lime requirement of the soil and that of acid phosphate the chemical equivalent to the normal application of the salts. For the sake of brevity, hereafter throughout the discussion, we will refer to these three different amounts as the normal, the minimum, and the maximum application.

The chemicals were thoroughly incorporated in the sand, and seeds of sweet clover were sown. In order to insure a sufficient number of good seedlings the seeds were sown rather freely, but as the plants grew they were gradually thinned out until finally only five plants were left in each pot. The plant-food solutions were prepared and applied as directed in Hopkins and Pettit's "Soil Fertility Laboratory Manual" (34). Two crops have been grown in this series. The first was planted on July 17 and harvested November 1, 1919; the second was planted on January 19, but because of some unknown causes the seedlings failed to attain a uniform stand, so the whole series was replanted on February 2. The crop was harvested on May 21. The yields for two crops are given in table 5.

During the first crop several things were observed which led up immediately to the setting up of another series, and to the introduction of some modifications in the treatment of the pots for the second crop. In the first place, it was noticed that on every pot receiving acid phosphate no plant would grow.

Since the application of acid phosphate was rather heavy, 964 pounds to the acre or 5.3 gm. per pot, it was thought that the failure of the plants to grow might have been due to excessive amounts of acid phosphate present rather than to the presence of aluminum; accordingly a new series with acid phosphate alone, and in combination with calcium carbonate, was set up. This was designated as 600 series. The results of this series are shown in plate 6. It proved the supposition true that acid phosphate in such amounts was injurious to seedlings. Even calcium carbonate in amounts sufficient to neutralize the acidity of the acid phosphate did not prevent the harmful effect of acid phosphate. With this experience the application of acid phosphate was reduced to from 100 to 400 pounds to the acre, in the second planting.

TABLE 5

Aluminum series (dry weight of five plants)

SERIES NUMBER	FIRST CROP[1]	SECOND CROP[2]	SERIES NUMBER	FIRST CROP	SERIES NUMBER	FIRST CROP	SECOND CROP	SERIES NUMBER	FIRST CROP	SECOND CROP	SERIES NUMBER	FIRST CROP
	gm.	gm.		gm.		gm.	gm.		gm.	gm.		gm.
101	3.40[3]	9.73	201	4.10	301	3.50	8.89	401	9.13	11.11	411	8.51
102	0.00	3.60	202	0.00	302	0.00	1.85	402	8.74	14.07	412	10.42
103	2.85	8.20	203	0.00	303	1.40	8.02	403	10.80	12.29	413	0.00
104	0.00	0.00	204	0.00	304	0.00	0.00	404	3.67	12.14	414	7.14
105	16.59	13.06	205	17.15	305	11.99	11.68	405	15.41	13.24	415	9.16
106	1.65	5.50	206	0.80	306	4.22	5.38	406	13.13	11.38	416	14.87
107	18.52	10.04	207	16.07	307	16.17	19.73	407	9.36	9.74	417	14.03
108	0.00	2.72	208	0.00	308	0.00	2.55	408	0.00	10.56	418	0.00
109	0.85	2.02	209	0.00	309	4.00	1.93	409	20.00	11.68	419	9.72
110	0.00	4.07	210	0.00	310	0.00	6.56	410	0.00	14.50		

[1] Harvested at the age of 106 days.

[2] Average of 2 pots.

[3] Harvested at the age of 108 days.

The second observation made was on the showing of the plants in the control pots of every series, except those of the aluminum monohydroxide series. The plants in these pots appeared to be suffering from lack of some elements. Since the plant-food solution applied to the pots did not contain calcium, it was thought that the plants in the controls might have been suffering from lack of calcium, in which case the results of the different treatments would not be comparable. Following this thought it was planned for the second crop to apply calcium silicate to each pot as a source of calcium, and in quantities having calcium equal to the amount contained in calcium carbonate applied as normal.

The third observation made was on the aluminum monohydroxide series in which all pots except those receiving the maximum amount of aluminum monohydroxide, and acid phosphate, show no effect of the presence of aluminum.

This compound, being insoluble, will produce no toxic effect, but it was thought that adding some substances which would yield acids on decomposition might change the aluminum hydroxide into a soluble form of aluminum, thus throwing further light on the form of aluminum compounds in the soil that produce toxicity. So it was planned for the next crop to introduce ammonium sulfate and dried blood in the series. Then the aluminum chloride series was dropped out in order to give way to this plan. Following is the plan of the experiments. Every treatment was carried out in duplicate.

PLAN OF THE EXPERIMENT

100 Series—Aluminum sulfate, $Al_2(SO_4)_3$

7.75 gm., or 3100 lbs. to the acre $Al_2(SO_4)_3 = 6.79$ gm. $CaCO_3$

5.3 gm., or 964 lbs. to the acre $CaH_4(PO_4)_2 = 7.75$ gm. $Al_2(SO_4)_3$ according to the following equation:

$$2Al_2(SO_4)_3 + CaH_4(PO_4)_2 = 2AlPO_4 + CaSO_4 + 2H_2SO_4$$
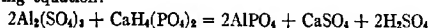
101. Control—Plant-food only
102. Plant-food + 7.75 gm. $Al_2(SO_4)_3$
103. Plant-food + ⅕, or 1.55 gm. $Al_2(SO_4)_3$
104. Plant-food + 5 × 7.75 gm., or 38.75 gm. $Al_2(SO_4)_3$
105. Plant-food + 7.75 gm. $Al_2(SO_4)_3 + 6.79$ gm. $CaCO_3$
106. Plant-food + 7.75 gm. $Al_2(SO_4)_3 + ⅕$, or 0.36 gm. $CaCO_3$
107. Plant-food + 7.75 gm. $Al_2(SO_4)_3 + 5 ×$, or 33.95 gm. $CaCO_3$
108. Plant-food + 7.75 gm. $Al_2(SO_4)_3 + 5.3$ gm. $CaH_4(PO_4)_2$
109. Plant-food + 7.75 gm. $Al_2(SO_4)_3 + ⅕$, or 1.06 gm. $CaH_4(PO_4)_2$
110. Plant-food + 7.75 gm. $Al_2(SO_4)_3 + 5 ×$, or 26.5 gm. $CaH_4(PO_4)_2$

200 Series—Aluminum chloride, $AlCl_3$

6.04, or 2405 pounds to the acre $AlCl_3 = 6.79$ gm. $CaCO_3$

5.3 gm., or 964 pounds to the acre $CaH_4(PO_4)_2 = 6.04$ gm. $AlCl_3$ according to the following equation:

$$2AlCl_3 + CaH_4(PO_4)_2 = 2AlPO_4 + CaCl_2 + 4HCl$$
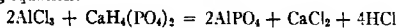
201. Control—Plant-food only
202. Plant-food + 6.04 gm. $AlCl_3$
203. Plant-food + ⅕, or 1.21 gm. $AlCl_3$
204. Plant-food + 5 ×, or 30.2 gm. $AlCl_3$
205. Plant-food + 6.04 gm. $AlCl_3 + 6.79$ gm. $CaCO_3$
206. Plant-food + 6.04 gm. $AlCl_3 + ⅕$, or 1.36 gm. $CaCO_3$
207. Plant-food + 6.04 gm. $AlCl_3 + 5 ×$, or 33.95 gm. $CaCO_3$
208. Plant-food + 6.04 gm. $AlCl_3 + 5.3$ gm. $CaH_4(PO_4)_2 CaCO_3$
209. Plant-food + 6.04 gm. $AlCl_3 + ⅕$, or 1.06 $CaH_4(PO_4)_2 CaCO_3$
210. Plant-food + 6.04 gm. $AlCl_3 + 5 ×$, or 26.5 $CaH_4(PO_4)_2 CaCO_3$

300 Series—Aluminum nitrate, $Al(NO_3)_3$

9.65 gm., or 3859 pounds to the acre $Al(NO_3)_3 \cdot 9H_2O × 6.79$ gm. $CaCO_3$

5.3 gm., or 964 pounds to the acre $CaH_4(PO_4)_2 = 9.65$ gm. $Al(NO_3)_3$ according to the following equation:

$$2 Al(NO_3)_3 + CaH_4(PO_4)_2 = 2AlPO_4 + CaNO_3 + 4HNO_3$$
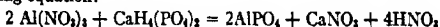
301. Control—Plant-food only
302. Plant-food + 9.65 gm. $Al(NO_3)_3$
303. Plant-food + ⅕, or 1.93 gm. $Al(NO_3)_3$

304. Plant-food + 5 ×, or 46.25 gm. Al(NO₃)₃
305. Plant-food + 9.65 gm. Al(NO₃)₃ + 6.79 gm. CaCO₃
306. Plant-food + 9.65 gm. Al(NO₃)₃ + ⅕, or 1.36 gm. CaCO₃
307. Plant-food + 9.65 gm. Al(NO₃)₃ + 5 ×, or 33.95 gm. CaCO₃
308. Plant-food + 9.65 gm. Al(NO₃)₃ + 5.3 gm. CaH₄(PO₄)₂
309. Plant-food + 9.65 gm. Al(NO₃)₃ + ⅕, or 1.06 gm. CaH₄(PO₄)₂
310. Plant-food + 9.65 gm. Al(NO₃)₃ + 5 ×, or 26.5 gm. CaH₄(PO₄)₂

400 Series—Aluminum Hydroxide, Al(OH)₃

3.5 gm., or 1399 pounds to the acre Al(OH)₃ = 6.79 gm. CaCO₃

7.8 gm. CaH₄(PO₄)₂, or 1418.5 pounds per acre, or 3.5 gm. Al(OH)₃ according to the following equation:

$$4 \, Al(OH)_3 + 3 \, CaH_4(PO_4)_2 = 4 \, AlPO_4 + Ca_3(PO_4)_2 + 12 \, H_2O$$
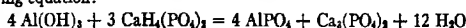
401. Control-Plant-food only
402. Plant-food + 3.5 gm. Al(OH)₃
403. Plant-food + ⅕, or 0.7 gm. Al(OH)₃
404. Plant-food + 5 ×, or 17.5 gm. Al(OH)₃
405. Plant-food + 3.5 gm. Al(OH)₃ + 6.79 gm. CaCO₃
406. Plant-food + 3.5 gm. Al(OH)₃ + ⅕, or 1.36 gm. CaCO₃
407. Plant-food + 3.5 gm. Al(OH)₃ + 5 ×, or 33.95 gm. CaCO₃
408. Plant-food + 3.5 gm. Al(OH)₃ + 7.8 gm. CaH₄(PO₄)₂
409. Plant-food + 3.5 gm. Al(OH)₃ + ⅕, or 1.5 gm. CaH₄(PO₄)₂
410. Plant-food + 3.5 gm. Al(OH)₃ + 5 ×, or 39.0 gm. CaH₄(PO₄)₂

600 Series—Acid phosphate, CaH₄(PO₄)₂

601. Control—Plant-food only
602. Plant-food + 5.3 gm. CaH₄(PO₄)₂
603. Plant-food + ⅕, or 1.06 gm. CaH₄(PO₄)₂
604. Plant-food + 5 ×, or 26.5 gm. CaH₄(PO₄)₂
605. Plant-food + 5.3 gm. CaH₄(PO₄)₂ + 6.79 gm. CaCO₃ ˙
606. Plant-food + 5.3 gm. CaH₄(PO₄)₂ + ⅕, or 1.36 gm. CaCO₃
607. Plant-food + 5.3 gm. CaH₄(PO₄)₂ + 5 ×, or 33.95 gm. CaCO₃

PLAN FOR SECOND CROP

100 Series. The same as before with the addition of 7.9 gm. CaSiO₃ to each pot and the reduction of the acid phosphate application to from 100 to 400 pounds to the acre, or from 350 mgm. to 1.00 gm. per pot.

300 Series. The same plan as before with the same modification noted in the 100 series.

400 Series. Up to 410, inclusive, the same plan as before with the same modification as noted in the 100 series.

From 411 to 424 the following arrangement has been followed:

From 411 to 417, inclusive, 4.4 gm. (NH₄)₂SO₄ has been added according to the following reaction:

$$(NH_4)_2SO_4 \rightarrow 2HNO_3 \text{ plus } H_2SO_4$$
$$\text{but } 3 \, HNO_3 \rightarrow Al(OH)_3$$
$$\text{and } 3H_2SO_4 \rightarrow 2 \, Al(OH)_3$$
$$\text{or } 132 \text{ gm. } (NH_4)_2SO_4 = 104 \, Al(OH)_3$$
$$\therefore \, 3.5 \text{ gm. } Al(OH)_3 = 4.4 \text{ gm. } (NH_4)_2SO_4$$
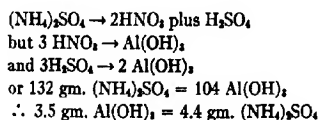
From 418 to 424, inclusive, 13.33 gm. of dried blood has been added, according to the ollowing reaction. The dried blood used contained 14 per cent N, or 17 gm. NH₃.

If all ammonia produced is nitrified, 2 HNO₃ is produced.

But $HNO_3 = Al(OH)_3$

∴ 189 gm. HNO₃ = 78 gm. Al(OH)₃

3.5 gm. Al(OH)₃ = 8.4 gm. HNO₃, or 13.33 gm. dried blood

411. Plant-food + 3.5 gm. Al(OH)₃ + 4.4 gm. (NH₄)₂SO₄ + 7.9 gm. CaSiO₃
412. Plant-food + 3.5 gm. Al(OH)₃ + ⅕, or 0.88 gm. (NH₄)₂SO₄ + 7.9 gm. CaSiO₃
413. Plant-food + 3.5 gm. Al(OH)₃ + 5 ×, or 0.22 gm. (NH₄)₂SO₄ + 7.9 gm. CaSiO₃
414. Plant-food + 17.5 gm. Al(OH)₃ + 4.4 gm. (NH₄)₂SO₄ + 7.9 gm. CaSiO₃
415. Plant-food + 3.5 gm. Al(OH)₃ + 4.4 gm. (NH₄)₂SO₄ + 7.9 gm. CaSiO₃
416. Plant-food + 3.5 gm. Al(OH)₃ + 4.4 gm. (NH₄)₂SO₄ + 7.9 gm. CaSiO₃
417. Plant-food + 3.5 gm. Al(OH)₃ + 4.4 gm. (NH₄)₂SO₄ + 7.9 gm. CaSiO₃
418. Plant-food + 3.5 gm. Al(OH)₃ + 13.33 gm. dried blood
419. Plant-food + 3.5 gm. Al(OH)₃ + ⅕, or 2.66 gm. dried blood
420. Plant-food + 3.5 gm. Al(OH)₃ + 5 ×, or 66.65 gm. dried blood
421. Plant-food + 17.5 gm. Al(OH)₃ + 13.33 gm. dried blood
422. Plant-food + 3.5 gm. Al(OH)₃ + 13.33 gm. dried blood
423. Plant-food + 3.5 gm. Al(OH)₃ + 13.33 gm. dried blood
424. Plant-food + 3.5 gm. Al(OH)₃ + 13.33 gm. dried blood

Results and discussion. The effect of aluminum salts, of aluminum hydroxide and of acid phosphate on the growth of sweet clover, is best shown in the photographs in plates 2, 3, 4, 5 and 6.

In the first crop in which no compound as a source of calcium was applied to the pots, aluminum sulfate proved to be injurious to sweet clover even in very small amounts. This may be seen in pots 102, 103 and 104 in which aluminum sulfate alone was applied. In pot 104, which received the maximum application of the salt, absolutely no seed could germinate. Pot 106, receiving the normal application of aluminum sulfate and one-fifth the normal application of calcium carbonate, shows that in small amounts calcium carbonate cannot correct the toxic effect of aluminum. On the other hand, where calcium carbonate has been applied in larger amounts, normal and maximum, sweet clover exhibited enormous growth. In the case of pots receiving aluminum sulfate and acid phosphate, those receiving 964 pounds and 4820 pounds of acid phosphate per acre failed to grow any crop.

What has been said about the effect of aluminum sulfate on sweet clover can also be said for aluminum chloride and aluminum nitrate. But with aluminum hydroxide the result is different. The normal application of aluminum hydroxide did not have any effect on sweet clover; the maximum, however, caused some depression. It is also important to note that whereas in the combination of aluminum salts and the minimum acid phosphate application, no sweet clover grew, but in that of the aluminum hydroxide and minimum acid phosphate no effect was shown. This proves that the failure of sweet clover to grow in pots 109, 209 and 309 was due to the presence of aluminum rather than that of acid phosphate.

The results of the second cropping, in which calcium silicate was added as a source of calcium, and the application of acid phosphate has been reduced to from 100 to 400 pounds per acre, were different from those of the first crop.

First, the normal application of aluminum sulfate did not show any toxic effect at all, while the maximum application was always fatal to sweet clover. Second, in every case where calcium carbonate was applied, no matter in what amount, good plants were growing, indicating that active aluminum has been put out of action. Acid phosphate in decreased amounts seemed to help in reducing the injurious effect of aluminum sulfate.

The results with aluminum nitrate were different from those noted in the case of sulfate. The normal application showed very toxic effects. While the maximum application of calcium carbonate was beneficial to clover the normal application did not entirely eliminate the toxicity of aluminum nitrate. The action of acid phosphate in eliminating the toxicity of aluminum nitrate was much less pronounced than in the case of the sulfate. From this difference of the behaviour of sweet clover on the two salts we are led to conclude that aluminum nitrate chemically equivalent to the acidity of the soil is more toxic than aluminum sulfate.

In the case of aluminum hydroxide, up to pot 419 with the exception of pots 413 and 419, the stand of sweet clover was uniform. Even the maximum application did not produce any effect on the growth of the plants. Pot 413 received 22 gm., the maximum application, of ammonium sulfate. The fact that sweet clover did not grow cannot be attributed to any cause but to an excessive amount of ammonium salt which, on breaking down, liberates ammonia that causes injury to the germinating seeds. Pot 418 received 13.33 gm., the normal application, of dried blood. Pot 419 received 2.66 gm., the minimum application, of dried blood. And the fact that on the former nothing grew, while on the latter the crop was as good as that in any other pot in the series, can be attributed also to the excessive amount of dried blood which on decomposition produces ammonia that hinders the germination of seeds. Apparently neither ammonium sulfate nor dried blood in smaller amounts was able to change aluminum hydroxide into other forms of aluminum which could produce the same effect as aluminum sulfate or nitrate.

The effect of aluminum salts on sweet clover in the presence of calcium carbonate. In the series with aluminum sulfate, chloride and nitrate we have noted that in the presence of an excess of calcium carbonate the toxicity of the salts was overcome. Ruprecht and Morse (60) found this to be true also in their water-culture investigation with aluminum and iron sulfates in which, whenever calcium carbonate was added in excess to the solution containing aluminum and iron, the toxicity of these metals has been eliminated and good healthy plants grew in the solution. With this fact the question naturally arises as to what became of the aluminum. The most logical conclusion would be that aluminum had entered into combination with other elements forming an insoluble compound. Ruprecht and Morse (60) suggested that aluminum was precipitated as hydroxide and as such had no effect on the plants grown in the solution. The results of the test carried out in this work proved that aluminum monohydroxide has no effect on sweet clover. But whether the

hydroxide precipitated by the introduction of calcium carbonate is the variety that does not dissolve in water, acid and alkali, the author seriously doubts. In fact, he is of the opinion that the aluminum hydroxide precipitated by the introduction of calcium carbonate is like that formed when ammonia and sodium or potassiuum hydroxide is added to a solution containing aluminum. This is aluminum trihydroxide, which is insoluble in water and would therefore have no effect on the plants grown in solution. But this form of hydroxide is soluble even in dilute acids (53), and in sand or soil where chemical changes are constantly taking place this form of aluminum hydroxide will not remain long, for it will be converted into aluminum sulfate, chloride and nitrate as fast as free sulfuric, hydrochloric and nitric acids are produced in the soil and as long as the soil is not supplied with calcium or other suitable bases. For this reason the author looks into the formation of a more stable aluminum compound as an explanation for the elimination of active aluminum when calcium carbonate is added to sand or soil. He is of the opinion that as soon as calcium bicarbonate is formed by the action of carbonated water on calcium carbonate, the bicarbonate reacts with the aluminum salts forming calcium aluminate. The reaction may be written as follows:

$$Al_2(SO_4)_3 + 3Ca(HCO_3)_2 = Al_2O_3.3CaO + CaSO_4 + 3H_2O.6CO_2$$
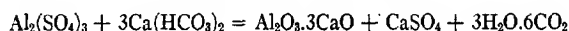
Calcium aluminate is one of the constituents of portland cement and is a very stable compound. The formation of this compound seems to be the only satisfactory explanation for the ineffectiveness of aluminum as a toxic substance in the presence of sufficient calcium carbonate.

The effect of aluminum salts on sweet clover in the presence of acid phosphate. Mention has been made before that the 964 pounds per acre application of acid phosphate proved to be detrimental to sweet clover, and that the failure of the crop in the pots which received the normal and maximum application was brought about by the excess of acid phosphate. But in the second crop where the acid phosphate was applied in reduced amounts from 100 to 400 pounds per acre, the results showed that acid phosphate reduced the toxicity of aluminum. While the pots receiving the minimum and normal application in series 300 did not show any reduction of the toxicity of aluminum nitrate, the reduction of toxicity in the pot receiving 400 pounds of acid phosphate per acre is very pronounced, as indicated by the fairly good growth of the plants. Evidently the minimum and normal applications were not sufficient to convert the larger portion of aluminum into an insoluble form. Now the question arises as to how acid phosphate reduced the toxicity of aluminum. The answer is that with acid phosphate, aluminum sulfate, chloride and nitrate form an insoluble compound. In this case the compound is aluminum phosphate and is formed according to the following reaction:

$$2Al(NO_3)_3 + CaH_4(PO_4)_2 = 2AlPO_4 + Ca(NO_3)_2.4HNO_3$$
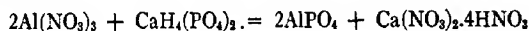
Under soil conditions this reaction is probably never complete; nevertheless, a great amount of insoluble aluminum phosphate is formed. But the free nitric acid formed might also react with more aluminum, thus repeating the process until equilibrium is finally reached. Aluminum phosphate is highly insoluble and Wheeler (82) thinks that in the case of acid soils it is desirable to apply lime before or at the same time with acid phosphate in order to prevent formation of aluminum phosphate which is even more insoluble than tricalcium phosphate.

Experiment II. Effect of limestone and acid phosphate alone and in combination on the productivity and acidity of acid soils

Ten duplicate pots for each type of soil were filled with about 5 kgm. (11 pounds) of soil, and treated according to the following plan:

PLAN OF THE EXPERIMENT

700 Series—Gray silt loam

Acidity = 4.94 gm. $CaCO_3$ per 5 kgm., or 5.3 gm. limestone of 93 per cent purity, or 2125 pounds to the acre.

    701. Control (nothing)
    702. Soil + 5.3 gm. limestone
    703. Soil + ⅕, or 1.0 gm. limestone
    704. Soil + 5 ×, or 26.5 gm. limestone
    705. Soil + 5.8 gm. $CaH_4(PO_4)_2$ = 1.05 tons to the acre
    706. Soil + ⅕, or 1.16 gm. $CaH_4(PO_4)_2$ = 0.21 tons to the acre
    707. Soil + 5 ×, or 29 gm. $CaH_4(PO_4)_2$ = 5.25 tons to the acre
    708. Soil + 5.8 gm. $CaH_4(PO_4)_2$ + 5.3 gm. limestone
    709. Soil + 5.8 gm. $CaH_4(PO_4)_2$ = ⅕, or 1.06 gm. limestone
    710. Soil + 5.8 gm. $CaH_4(PO_4)_2$ = 5 ×, or 26.5 gm. limestone

800 Series—Yellow gray silt loam

Acidity = 6.5 gm. $CaCO_3$ per 5 kgm., or 2813 pounds limestone to the acre

    801. Control
    802. Soil + 7.03 gm. limestone
    803. Soil + ⅕, or 1.40 gm. limestone
    804. Soil + 5 ×, or 35.15 gm. limestone
    805. Soil + 7.6 gm. $CaH_4(PO_4)_2$ = 1.4 tons to the acre
    806. Soil + ⅕, or 1.5 gm. $CaH_4(PO_4)_2$ = 0.28 tons to the acre
    807. Soil + 5 ×, or 38.0 gm. $CaH_4(PO_4)_2$ = 6.00 tons to the acre
    808. Soil + 7.6 gm. $CaH_4(PO_4)_2$ + 7.03 gm. limestone
    809. Soil + 7.6 gm. $CaH_4(PO_4)_2$ + ⅕, or 1.40 gm. limestone
    810. Soil + 7.6 gm. $CaH_4(PO_4)_2$ + 5 ×, or 35.15 gm. limestone

900 Series—Yellow silt loam

Acidity + 6.79 gm. $CaCO_3$ per kgm., or 7.4 gm. limestone, or 2921 pounds limestone to the acre.

    901. Control.
    902. Soil + 7.3 gm. limestone
    903. Soil + ⅕, or 1.46 gm. limestone

904. Soil + 5 ×, or 36.5 gm. limestone
905. Soil + 7.6 gm. CaH₄(PO₄)₂, or 1.4 tons to the acre
906. Soil + ⅕, or 1.58 gm. CaH₄(PO₄)₂, or 0.28 tons to the acre
907. Soil + 5 ×, or 39 gm. CaH₄(PO₄)₂, or 6.00 tons to the acre
908. Soil + 7.8 gm. CaH₄(PO₄)₂ + 7.3 gm. limestone
909. Soil + 7.8 gm. CaH₄(PO₄)₂ + ⅕, or 1.46 gm. limestone
910. Soil + 7.8 gm. CaH₄(PO₄)₂ + 5 ×, or 36.5 gm. limestone

Two crops have been harvested in these series. The first was planted on August 22, 1919, and harvested November 30, 1919, a period of only 100 days. The time of cropping could have been prolonged, but when the crop was moved into the greenhouse, the red spider infested it so badly that it was thought best to cut the crop down in order to eradicate the red spider at once. The second crop was planted on January 16, 1920, and harvested May 21, 1920, after a period of 125 days. The yield of the crops is given in table 6.

TABLE 6

Soil series (dry weight of five plants)

GRAY SILT			YELLOW GRAY SILT			YELLOW SILT		
Series number	First crop[1]	Second crop[3]	Series number	First crop	Second crop	Series number	First crop	Second crop
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
701	0.54[2]	2.02	801	0.27	0.40	901	0.52	0.79
702	0.81	2.77	802	0.68	2.29	902	0.64	1.26
703	0.70	2.07	803	0.26	0.26	903	0.52	1.29
704	1.28	5.35	804	0.97	3.45	904	1.41	2.11
705	0.87	2.20	805	0.40	0.60	905	0.75	4.72
706	0.58	1.62	806	0.23	1.68	906	0.62	1.38
707	1.12	3.18	807	0.67	1.48	907	1.21	1.45
708	1.65	5.88	808	0.71	10.58	908	1.19	4.67
709	0.76	1.58	809	0.34	1.80	909	0.71	1.10
710	1.43	7.42	810	1.45	14.67	910	1.66	6.49

[1] Harvested at the age of 106 days.

[2] Average of 2 pots.

[3] Harvested at the age of 108 days.

For each type of soil a corresponding set of treated pots was laid aside, without plants for acidity determination. Two determinations were made, the first on December 8, 1919, after a lapse of 108 days from the time the pots were set aside, and the second on February 16, 1920, a period of 70 days after the first determination. Table 7 shows the results of these determinations.

Results and discussion. The effect of lime and acid phosphate on sweet clover grown on acid soils can best be seen in the photographs in plates 6, 7, and 8. It can be seen that all the three types of soil respond to liming. The normal and maximum applications especially brought excellent results. The plants were healthy, vigorous and dark green. Limestone applied in amounts equal to one-fifth of the lime requirement did not benefit the soil at all. The growth of the plants in this case was comparable to that of the control in which

TABLE 7

Acidity determinations of treated soils

SERIES NUMBER	SAMPLED DECEMBER 8, 1919				SAMPLED FEBRUARY 16, 1920				
	P.p.m.	Acidity reduced after 108 days	Acidity above or below control	Acidity reduced due to treatment	P.p.m.	Acidity reduced after 178 days	Acidity above or below control	Acidity reduced between dates of sampling	Acidity reduced due to treatment

Gray silt loam. Original acidity—988 parts per million, or 2125 pounds of limestone per acre

		per cent	per cent	per cent		per cent	per cent	per cent	per cent
701	705	28.66			685	30.87		2.83	
702	165	83.29	76.59	54.63	74	92.51	89.19	55.15	62.64
703	640	35.22	9.22	6.56	425	56.98	37.95	33.59	26.11
704	26	97.26	96.31	68.60	Alkaline	100.00	0.00	0.00	
705	545	44.83	22.69	16.17	217	78.03	68.32	41.83	47.16
706	810	18.01	12.96		687	30.46	0.29	15.18	
707	467	52.85	33.75	24.19	115	88.35	83.19	75.37	57.48
708	106	89.27	84.96	60.61	Alkaline	100.00	0.00	0.00	
709	437	56.78	38.96	21.12	243	75.40	64.52	44.39	44.53
710	20	97.97	96.32	69.31	Alkaline	100.00	0.00	0.00	

Yellow gray silt loam. Original acidity—1358 parts per million, or 2813 pounds of limestone per acre

801	1066	21.63			970	28.57		9.00	
802	42	96.90	96.06	75.27	Alkaline	100.00	0.00	0.00	
803	542	60.09	49.06	38.46	362	73.34	61.64	33.21	44.7
804	Alkaline	100.00	0.00		Alkaline	0.00	0.00	0.00	
805	764	43.00	28.33	21.37	580	57.29	40.20	24.08	28.62
806	895	33.28	16.04	11.65	894	34.16	0.11	5.83	5.59
807	479	63.91	55.09	42.28	271	80.04	72.06	41.33	51.47
808	110	91.91	90.62	70.28	Akaline	100.00	0.00	0.00	
809	565	65.75	46.43	44.12	519	61.78	46.49	8.01	33.21
810	Alkaline	100.00	0.00	0.00	Alkaline	100.00	0.00	0.00	

Yellow silt loam. Original acidity—1318 parts per million, or 2921 pounds of limestone per acre

901	685	48.02			683	48.18		0.29	
902	77	94.16	88.76	46.13	13	99.01	98.09	83.11	50.83
903	511	61.22	25.40	13.20	243	81.56	64.42	52.44	33.38
904	Alkaline	0.00	0.00		Alkaline	0.00	0.00	0.00	
905	630	52.20	8.26	4.18	441	65.78	35.43	30.00	17.60
906	832	36.71	17.66		645	51.06	5.56	22.47	2.88
907	455	65.47	33.57	17.45	123	90.66	81.99	72.96	52.48
908	109	91.72	84.09	43.70	Alkaline	100.00	0.00	0.00	0.00
909	846	35.81	19.05		642	51.29	6.00	24.11	3.11
910	16	98.78	96.21	50.76	Alkaline	100.00	0.00	0.00	

the plants were very small and chlorotic. The results with acid phosphate applied alone, showed that the soils also respond to phosphate fertilization. Judging from the growth of the plants even the minimum application seems to have benefited the soil. In the first crop, however, the plants looked different from those growing on limed pots. The plants grew more than those in the control, but they were slender, branchless, and chlorotic as compared with the bushy dark green plants growing on the limed pots. In the second crop, excepting the crops in the pots which received the minimum application, those defects observed above have disappeared, and although growth was slow during the winter days, the plants were healthy, bushy and deep green. The best crop in this series was noted in the pots receiving the normal application and maximum application of limestone, together with the normal application of acid phosphate. Even the minimum application of limestone in combination with the normal amount of acid phosphate grew better crops than the normal application of limestone alone. In the first crop, however, the plants were also chlorotic, although to a lesser extent than those in pots receiving acid phosphate alone. In the second crop chlorosis has completely disappeared.

After the first acidity determination it was found that the acidity of the untreated soil has been reduced also. The acidity of the gray silt loam has been reduced 28 per cent, that of the yellow gray silt $21\frac{1}{2}$ per cent and that of the yellow silt 48 per cent. Up to this time, for a period of 108 days, tap-water was used for watering the plants and the pots, but since then rain-water was used instead. Experience in the use of this tap-water in the greenhouse proved that it has a tendency to reduce the acidity of acid soils. For example, a very acid soil watered by the tap-water became alkaline after a few years. The fact that the acidity of the controls of the three types of soil have been reduced is attributed to the use of the tap-water. But by subtracting the per cent of acidity reduced in the control from the total acidity of the treated pots, we still have a fair indication of the acidity reduced due to the treatment of the soils.

The effect of limestone and acid phosphate alone and in combination on the acidity of the three acid soils is best shown in plates 11 and 12, in which the treatment of the pots is represented by the abscissas and the per cent of acidity reduced by the ordinates. Curve 1 in each figure represents the per cent of acidity reduced after 108 days and curve 2 the total acidity reduced for a period of 178 days.

The three types of soil responded differently to the different treatments. Of the three the yellow gray silt loam responded more readily to liming and phosphate fertilization than either of the other two types. From the charts we can see that in 108 days the normal application of limestone or the amount required to neutralize the acidity of the soil reduced the acidity of the gray silt about 55 per cent, of the yellow silt 46 per cent and that of the yellow gray silt 76 per cent. In 178 days the total acidity reduced was 63 per cent for the gray silt and 51 per cent for the yellow silt; the yellow gray silt was com-

pletely neutralized, the reaction being alkaline. With one-fifth of the normal application the acidity was reduced 6.56 per cent in the case of gray silt, in 108 days; 38 per cent in case of the yellow gray, and 13 per cent in case of the yellow silt. At the end of 178 days the lime applied was completely used up in the neutralization of one-fifth of the acidity of the soil. In the case of the yellow gray silt the percentage of acidity reduced in both determinations exceeds that which would theoretically be accomplished by lime applied in an amount equal to one-fifth of the lime requirement. This fact is probably due to experimental error which would include sampling and manipulation. When limestone equal to five times the lime requirement was added, the neutralization of acidity was complete in 108 days in the case of two soils; only 68.8 per cent of the acidity was reduced in the case of gray silt. All these facts indicate the rapidity with which calcium carbonate puts active aluminum out of action, the substance responsible for the acidity of the soils.

The effect of acid phosphate on the three soils is interesting. The notion that acid phosphate has the tendency to increase the acidity of a soil has no confirmation in this work. On the contrary, the results show that acid phosphate decidedly reduced the acidity of the soil, as measured by the Hopkins method. The reaction, however, is slower than that in the case of calcium carbonate. In 108 days the normal application of acid phosphate destroyed 16 per cent of the acidity of the gray silt; 21 per cent of that of the yellow gray silt; and 4 per cent of that of the yellow silt. At the end of 178 days 47 per cent of the acidity of the gray silt was destroyed; 28 per cent of that of the yellow gray silt and only 17 per cent of that of the yellow silt. Applied in one-fifth the normal application, acid phosphate reduced in 178 days the acidity of the gray silt 7 per cent; of the yellow gray silt 12 per cent; and of the yellow silt only 3 per cent. In five times the normal application acid phosphate reduced the acidity of the gray silt 24 per cent in 108 days and 57 per cent in 178 days; of the yellow gray silt 42 per cent in 108 days and 51 per cent in 178 days; of the yellow silt 17 per cent in 108 days and 52 per cent in 178 days.

The combination of acid phosphate and limestone produced a still more interesting result. The combination of the normal application of limestone and acid phosphate reduced, in 108 days the acidity of the gray silt 60 per cent; of the yellow gray silt 70 per cent; and of the yellow silt 43 per cent. After 178 days all the pots with this treatment were alkaline. The combination of the normal application of acid phosphate and one-fifth the normal dose of limestone also reduced considerably the acidity of the soils. But with the combination of the normal application of acid phosphate and the maximum of limestone, the yellow gray silt was alkaline in 108 days, while the gray and yellow silt were then reduced 69.31 per cent and 50.76 per cent, respectively. At the end of 178 days the soils were alkaline.

How acid phosphate reduces the acidity of acid soils. One of the problems in the present investigation is whether acid phosphate increases the acidity of an acid soil. Using the calcium-acetate method Hartwell and Pember (27) found

that the acidity of acid soils increases as the amount of acid phosphate applied was increased. Comparing the lime-water and the potassium-nitrate methods Albrecht[1] found also that with the lime-water method the acidity increased as the amount of acid phosphate was increased, but with the potassium-nitrate method up to a certain point, the increase of acid phosphate was accompanied by a decrease of acidity. The results discussed in the preceding paragraph corroborate the findings of Albrecht with the potassium-nitrate method. In this connection two questions come up. First, if acid phosphate reduced the acidity of the soil, how? And second, why are the results between the potassium-nitrate method on the one hand, and those of calcium-acetate and lime-water on the other, so diametrically opposed?

Hartwell and Pember (27) noted that while the acidity of the soils was increased with the increase of acid phosphate application the amount of active aluminum was decreased. No explanation was offered for this fact, but we can safely attribute it to the combination of active aluminum with other elements forming an insoluble compound. One of the products of the reaction between acid phosphate and active aluminum in the soil is aluminum phosphate, a very insoluble compound. The decrease in the amount of active aluminum after acid phosphate has been applied to the soil is, therefore, due to the formation of aluminum phosphate. The larger the amount of acid phosphate applied to the soil containing active aluminum, the larger will be the amount of aluminum phosphate formed. And since aluminum is largely responsible for the acid reaction of the potassium nitrate extract, the larger the amount of aluminum converted into phosphate, the smaller will be the amount of aluminum that will be brought into solution when an acid soil is extracted with potassium nitrate after acid phosphate has been applied. This explains the fact that with the potassium-nitrate method the acidity of acid soils decreases as the amount of acid phosphate applied increases.

We can see from the above explanation that the opposing results obtained by the use of the three methods of determining the acidity of the soil are due to the difference of the substances determined. The lime-water and calcium-acetate methods determine true acidity, and the potassium-nitrate method, while originally intended to determine true acidity, actually determines active aluminum. Since acid phosphate has some free phosphoric acid the first two methods will record increased acidity as the amount of acid phosphate is increased.

Hartwell and Pember (27) also observed that in spite of the large amount of acidity (as determined by calcium acetate) due to acid phosphate, barley made a marked growth. In the present investigation the growth of sweet clover on pots receiving acid phosphate alone increased as the acid phosphate applied increased. But the correlation is between growth and decrease of acidity rather than growth and increase of acid phosphate. This is better

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[1] W. Albert Albrecht's unpublished work in the University of Illinois.

illustrated in table 8 in which the per cent of acidity reduced and the dry matter of five plants from each pot are put together.

The results given are one of the evidences that aluminum is an important factor in the acidity of the three types of soil studied.

TABLE 8

Effect of the reduction of acidity by acid phosphate on the yield of sweet clover

Gray silt

Pot number......................	70i	705	706	707
Per cent of acidity reduced........		16.16	5.70	24.19
Dry weight (gm.)................	0.54	0.87	0.58	1.12

Yellow gray silt

Pot number.....................	801	805	806	807
Per cent of acidity reduced........		21.37	11.65	42.28
Dry weight (gm.)................	0.27	0.40	0.23	0.67

Yellow slit

Pot number.....................	901	905	906	907
Per cent of acidity reduced........		4.18	1.36	17.45
Dry weight (gm.)................	0.52	0.75	0.62	1.21

Experiment III.   What happens when acid soils are leached out with potassium nitrate or water

Two pots of each of the three types of soil were leached out with normal potassium nitrate until the last 125 cc. of leachings were practically neutral. With the gray silt loam 30 liters of the solution per pot were needed to reach this point.   For the yellow gray silt loam 35 liters were needed, and for the yellow silt 39 liters.   After leaching with potassium nitrate the soil was leached out with distilled water again in order to get rid of the excess of potassium nitrate.   The leaching was continued also until the last few drops showed faintly blue to the diphenylamine sulfuric acid test for nitrates.   Then the soils were dried out and sampled for analysis.   The results of the analysis are given in column 3, table 4.

A similar set was leached out with water alone.   Distilled water was used in leaching the soils and the operation was continued until the last 125 cc. needed hardly 0.5 cc. of the standard sodium hydroxide solution used in titrating the acidity.   When this end was reached, 12 liters of water had been used in the gray silt; 16 liters in the yellow gray silt; and 13 liters in the yellow silt.   Then the soil was dried and sampled as in the above set for analysis. The results of the analysis are given in column 5, table 4.

What has been found from the above experiments may be summarized as follows.   With potassium nitrate 96.96 per cent of the acidity of the gray silt

was extracted, with water only 7.31 per cent of the acidity was extracted. Of the aluminum 44.79 per cent was leached out by potassium nitrate and 17.67 per cent by water; of the iron 23.85 per cent was leached out by potassium nitrate and 7.53 per cent by water; of the manganese 4.76 per cent and 1.19 per cent were leached out by potassium nitrate and water, respectively. Potassium increased 28.95 per cent and nitrate nitrogen 20.69 per cent.

With the yellow gray silt 99.15 per cent of the acidity was extracted by potassium nitrate and 7.21 per cent by water; 59.93 per cent of the aluminum was leached out by potassium nitrate and 24.73 per cent by water; 14.44 per cent of the iron was leached out by potassium nitrate and 2.48 per cent by water; and of manganese 3.03 per cent and 1.03 per cent was leached out by potassium nitrate and water, respectively. Potassium was increased 25.72 per cent and nitrate nitrogen 55.60 per cent.

With the yellow silt, potassium nitrate extracted 97.93 per cent of the acidity, and water 12.36 per cent; of aluminum 50.61 per cent was leached out by potassium nitrate and 21.55 per cent by water; of iron 21.01 per cent was leached out by potassium nitrate and 8.62 per cent by water; of manganese 8.79 per cent was extracted by potassium nitrate and 1.51 per cent by water. Potassium was increased by 28.57 per cent and nitrate nitrogen by 90.47 per cent. It may be added that potassium nitrate also leached out some of the calcium and phosphorus of the soils.

Discussion of results. These results reveal the fact that from 44 to 60 per cent of the aluminum in the soil may be leached out by potassium nitrate and that the leaching of this amount is accompanied by a big decrease in the acidity. Thus the 44.79 per cent of aluminum leached out from the gray silt was accompanied by the disappearance of 96.96 per cent of the acidity. In the case of the yellow gray silt the extraction of 59.93 per cent was accompanied by the destruction of 99.15 per cent of the acidity. With the yellow silt 50.61 per cent of the aluminum extracted was equivalent to a 97.93 per cent decrease in the acidity. It is not to be expected to extract all the aluminum in order to reduce the acidity of the soil to zero, for not all the aluminum in the soil is in the form readily soluble in potassium-nitrate solution. Some of the aluminum is present as silicate and since clay constitutes from 10.15 to 26.4 per cent of the bulk of the soils under experiment, it is not unlikely that kaolinite, $Al_2(OH)_4Si_2O_5$, the chief constituent of clay, is present in considerable amounts. Kaolinite is a very stable compound, and although kaolin (70), a mechanical mixture of kaolinite and silica, has been found to exchange bases with salt solutions, nevertheless, under the conditions in which the aluminum has been leached out in the present work, it is not probable that kaolinite and allied aluminum minerals will be readily attacked by potassium nitrate solutions. The case is more likely to be that a considerable amount of soluble aluminum compounds—salts and the trihydroxides—are present in the soil. In contact with potassium nitrate or even with water these compounds readily go into solution and are leached out. The 40 or 60 per cent of aluminum

leached out represents these soluble compounds or active aluminum. This active aluminum is equivalent approximately to 53,240 and 90,720 pounds per acre, respectively.

In the case of the water-leached soils it is seen that from 17.67 per cent to 24.73 per cent of the aluminum is leached out. These percentages are equivalent approximately to 20,570 pounds and 26,388 pounds per acre, respectively. The quantity of aluminum found in the water leachings would not probably be the actual amount of soluble aluminum in the field because certain factors, such as the transporting, storing and drying of the soil when brought to the greenhouse, might have contributed to the increase of the solubility of aluminum, but allowing 50 per cent to these factors we have still about 10,000 or 18,000 pounds left to be assigned to the readily soluble aluminum in the soils. The amount of aluminum in the maximum application of aluminum nitrate in the sand series is equivalent to 2701 pounds only, and this proved fatal to sweet clover. In the normal application of the same salt the aluminum is equivalent to 540 pounds per acre only, yet this proved toxic to sweet clover. If this is true what a tremendous influence will 10,000 or 18,000 pounds have on the crop in the field.

Sweet clover on the leached-out soils. Sweet clover seeds were sown in both potassium-nitrate-leached and water-leached soils. In the case of the former there were some difficulties which were never overcome in the case of two soils up to the writing up of this work. First, too much potassium nitrate was left in the soils in spite of the leaching by water. Gray silt loam had 28.69 per cent more nitrate nitrogen and 28.95 per cent more potassium than the original. Yellow gray silt loam had 55 per cent more nitrate nitrogen, and 25.72 per cent more potassium than the original; and the yellow silt had 90.27 per cent more nitrate nitrogen and 28.57 per cent more potassium. The second difficulty was the physical texture of the soils which was badly affected by the leaching with potassium nitrate. The soils became more compact and sticky. The first planting was consequently a failure. An attempt has been made to improve the physical texture of the soil by mixing the soil with one-third of its volume of pure silica sand and leaching out with water again. But the nitrate remaining was still in large enough amounts to be fatal to seedlings, consequently the second planting was again a failure. The soils were laid aside and watered every day until it was thought enough nitrate salt had been drained out. Seeds were then planted. At the beginning the seedlings seemed to be making headway, but within three weeks the seedlings in the yellow gray and yellow silt loams were already either dead or dying. Evidently the concentration of salts in these two types of soil was still too strong for the plants to survive. In the case of the gray silt the seedlings persisted and grew, although slowly. The growth up to May 19, at the age of 120 days, is shown in plate 9. A, is water-leached soil, A-1 is potassium-nitrate-leached soil, A-2 original soil plus KNO$_3$ equivalent to the excess found in the KNO$_3$-leached soil plus sand, A-3, the original soil plus sand. Attention

is called to the difference of the growth of sweet clover on the different pots. The plants in the potassium-nitrate-leached soil, although somewhat stunted in growth, were really healthy and deep green. The plants in A-2 and A-3 were largely chlorotic. This difference in the growth is attributed to the reduction of 96 per cent of the acidity of the soil, which is equivalent to 53,240 pounds of aluminum removed. It is also important to note the growth of sweet clover on the water-leached soil. Although only 96 days old they looked just as vigorous as those in the potassium-nitrate-leached soil. This was due chiefly to the presence of a still excessive amount of the nitrate salt in the potassium-nitrate-leached soil. But it is evident that the removal of about 20,000 pounds of aluminum by water had greatly benefited the growth of sweet clover and this amount was probably the amount of active aluminum immediately concerned in the unproductivity of the soils under investigation. This effect of the removal of about 44 per cent of aluminum in the soil by potassium nitrate and 17 per cent by water, on the growth of sweet clover, is conclusive proof that aluminum is the chief factor in the unproductivity of the three types of soil under investigation and probably of most acid soils in America.

How aluminum salts arise in the soil. The form of aluminum immediately concerned in the behaviour of sweet clover toward acid soils is the soluble form, the salts. The silicates and hydroxides cannot produce toxicity inasmuch as they are insoluble in water. In the case of one form of hydroxide, the monohydroxide, it has been proven harmless to sweet clover in the present investigation. But the salts have been proven injurious to plants even in dilute solutions.

The question now arises as to how aluminum salts may be formed in the soil. Aluminum chloride, sulfate and nitrate may all be found in the soil. The amount of aluminum chloride will naturally be limited by the absolute amount of chlorine in the soil. Aluminum sulfate will also be limited by the amount of sulfur. Under certain conditions, if the soil is rich in sulfur and the sulfur bacteria are active, through sulfofication considerable amounts of aluminum sulfate may be formed. Investigating the effect of sulfofication on the availability of potassium in the soil, Ames and Boltz (2) found that aluminum was not present in the extract of soils in which sulfur did not enter as a part of the treatment, and concluded that aluminum sulfate is formed during sulfofication. Artificial treatment of the soil may give rise to considerable amounts of aluminum sulfate. Ruprecht and Morse (60) found that the continuous application of ammonium sulfate to plots in the experimental field in Massachusetts produced aluminum sulfate. But great as is the possibility of the formation of aluminum sulfate in large quantities, still greater is the possibility for the formation of aluminum nitrate. Nitrification is a normal process occurring in the soil, and depending on conditions it varies widely. At certain seasons of the year nitrification is most active. Such is the condition under which large quantities of aluminum nitrate may be formed. In

normal soils, sufficiently provided with limestone, aluminum salt may never be formed, but in soils deficient in limestone, aluminum salts are largely formed; especially is it true when nitrification is most active. The acid-soluble aluminum trihydroxide in the soil, in the absence of limestone and other suitable bases, unites with nitric acid forming aluminum nitrate. The reaction may be represented by the following equation:

$$Al_2O_3.3H_2O + 6HNO_3 = 2Al(NO_3)_3 + 6H_2O$$
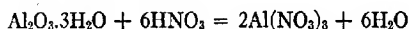
If limestone is present in sufficient quantities to satisfy the basic need of nitric acid produced, aluminum nitrate and sulfate may never be formed. Calcium nitrate, the best form of nitrogen compound for plant-food is formed, instead, according to the following reaction:

$$CaCO_3 + HNO_3 = Ca(NO_3)_2 + H_2O + CO_2$$
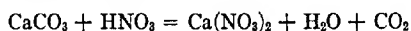
Ames and Boltz (2) noted that the largest amount of aluminum was found in solutions from soils where sulfur was oxidized in the absence of calcium carbonate.

What happens when acid soils are extracted with potassium nitrate before and after the application of limestone. The fact has been repeatedly observed in the present investigation that when an acid soil was extracted with potassium nitrate the reaction of the extract was always acid, but when limestone (calcium carbonate) was applied in amounts equivalent to or five times the lime requirement, and the soil was extracted with potassium nitrate the reaction of the extract was always alkaline and the white gelatinous aluminum hydroxide precipitate was absent. Knight[2] found that "when a base is added to an acid soil, comparatively insoluble products are formed. Calcium produces a product less soluble than does potassium." Ames and Boltz (2) also found that the addition of calcium carbonate at the rate of 80,000 parts per million on the soil, decreased the solubility of aluminum to 68 parts per million as compared with 660 parts per million where calcium was added in amounts just sufficient to combine with only a small part of the sulfuric acid. What most probably happens is this: When calcium carbonate is applied to the soil, calcium bicarbonate is formed which unites with the aluminum salts or with the acid-soluble hydroxide, forming calcium aluminate, a stable compound. The reaction may be written as follows:

$$Al_2(NO)_3.9H_2O + 6Ca(HCO_3)_2 = Al_2O_3.3CaO + 3Ca(NO_3)_2 + 15H_2O + 6CO_2$$

or

$$. Al_2O_3.3H_2O + 3Ca(HCO_3)_2 = Al_2O_3.3CaO + 6H_2CO_3$$
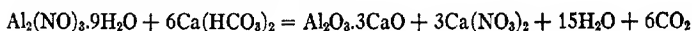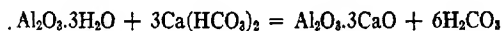
When an acid soil comes in contact with potassium nitrate solution an exchange of bases between the soil and the solution takes place (70). The

[2] H. G. Knight, "Acidity and Acidimetry of Soil," unpublished thesis from the University of Illinois.

aluminum compounds are attacked, bringing aluminum into solution and forming aluminum nitrate which on hydrolysis produces strong acidity. This is the cause of the acid reaction of the solution. When an acid soil is treated with calcium carbonate and after a while extracted with potassium nitrate the extract is alkaline. An exchange of bases takes place also. But in this case the calcium compounds formed in the soil are attacked by the salt solution, calcium being replaced by potassium and brought into solution as calcium nitrate. Such an explanation is in agreement with the findings of van Bemmelen (5) and other investigators (70) on the subject of exchange of bases between soils and salt solutions, the former having found that when potassium chloride solution was added to the soil, almost a complete change of potassium for calcium and magnesium took place. The presence of calcium nitrate, therefore, which does not hydrolyze, explains the neutral or alkaline reaction of the extract.

Experiment IV. Iron and manganese as factors in soil acidity

The table of analysis reveals that the types of soil under investigation also contain considerable quantities of iron and manganese. The gray silt contains 47,800 pounds of iron and 840 pounds of manganese per acre. The yellow gray silt contains 40,300 pounds of iron and 786 pounds of manganese per acre; and the yellow silt contains 74,200 pounds of iron and 660 pounds of manganese per acre. The toxicity of normal iron salts at a certain concentration is well known, and Ruprecht and Morse (61) found that in the unproductivity of ammonium-sulfate fertilized plots of the Massachusetts Agricultural Experiment Station, ferric and manganese salts were also contributing factors. Funchess (20) also observed that in Alabama the development of soluble manganese salts was the cause of the unproductivity of a certain soil. The question now comes up as to whether iron and manganese might not be also contributing factors in the acidity of the soils under investigation. It was thought that if these metals were as important a factor as aluminum, some idea might be obtained from their degree of solubility and their ratio to soluble aluminum. Fortunately, the first 4-liter potassium-nitrate leachings of the gray silt and the first 4-liter water-leachings of the three types of soil have been saved. These leachings were analyzed for aluminum, iron and manganese. The results of the analysis are given in table 9.

This table shows that the ratio of aluminum, iron and manganese in the first 4 liters of the potassium-nitrate leachings is 4.6:1:1.2. The first 4 liters of water extract gave for the gray silt loam 4.8 for aluminum, 1 for iron and 1.1 for manganese; for the yellow gray silt loam 6.2 for aluminum, 1 for iron and 1.3 for manganese; and for the yellow silt 3.4 for aluminum, 1 for iron and 1.7 for manganese. The proportion of aluminum to iron or manganese is such that there can be no doubt that aluminum is the dominant factor. In the case of manganese a further step has been taken. Sand-culture experiments were

carried out with manganese sulfate, nitrate and carbonate, the plan being the same as that in the aluminum series. The results of these experiments reveal that manganese cannot be any factor in the soil in question for this reason. When calcium carbonate in considerable quantities was combined with aluminum salts the toxicity of the aluminum was corrected. In the case of the soils, calcium carbonate also corrected their acidity or unproductivity, but with manganese compounds not even the application of five times the lime requirement of calcium carbonate has corrected the toxicity of manganese. It is not denied that iron and manganese might become factors in the acidity of some soils but with the soils under investigation there is no doubt that aluminum is the determining factor in their acidity.

TABLE 9

Analysis of extracts (4 liters)

TYPE OF SOIL	DETERMINED		
	Aluminum	Iron	Manganese
	KNO₃ extract		
Gray silt (mgm.)	282.5	60.9	49.8
Ratio	4.6	1	1.2
	H₂O extract		
Gray silt (mgm.)	75.5	15.8	14.6
Ratio	4.8	1	1.1
Yellow gray silt (mgm.)	105.2	17.0	13.1
Ratio	6.2	1	1.3
Yellow silt (mgm.)	88.9	26.3	16.3
Ratio	3.4	1	1.7

V. SUMMARY

Experiments have been carried out, first, to find out the influence of aluminum salts and aluminum hydroxide, alone and in combination with calcium carbonate or with acid phosphate, on the growth of sweet clover grown in sand; second, to determine the effect of limestone and acid phosphate alone and in combination, on the productivity and acidity of three types of silt loam soil; third, to find out the effect of the removal of some aluminum from the soil on the growth of sweet clover; and fourth, to ascertain whether iron and manganese also are factors in the acidity of the soils under investigation.

In the absence of some calcium compounds as a source of calcium, aluminum salts were highly toxic to sweet clover when applied in amounts chemically equivalent to the acidity of the soil, and fatal to sweet clover when applied in

amounts chemically equivalent to five times the acidity of the soil. In the presence of calcium silicate, aluminum nitrate was more toxic than aluminum sulfate.

Aluminum mono-hydroxide had no effect whatever on the growth of sweet clover, when other plant-food elements were added in water-soluble form.

Calcium carbonate in sufficient amounts corrected the toxicity of aluminum salts, by precipitating aluminum as calcium aluminate, an insoluble compound.

Acid phosphate applied at the rate of 400 pounds per acre reduced the toxicity of aluminum salts by forming aluminum phosphate, an insoluble aluminum compound.

Limestone applied at a rate equal to the lime requirement produced good crops on the three silt loam soils; applied at the rate of five times the lime requirement it produced better crops. At the same rate of application the soils were alkaline at the end of 178 days. The action of calcium carbonate in the soil is to unite with the aluminum salts and the acid-soluble aluminum hydroxide, forming calcium aluminate.

Acid phosphate applied alone at the rate of 1 ton to the acre produced fair crops, at the rate of 5 tons good crops. Acid phosphate also reduced the acidity of the soils and the decreases depended on the rate of application. At the rate of 5 tons per acre acid phosphate reduced the acidity of the soils from 51 to 57 per cent. The reduction of the acidity was due to the formation of the insoluble aluminum phosphate.

The combination of acid phosphate and limestone in large quantities produced the best crops.

The aluminum in the soil varies from 121,000 to 151,000 pounds per acre. When the soil was leached out with potassium nitrate solution until the last 125 cc. of leachings was practically neutral, the acidity of the soil was reduced 99 per cent and as much as 59 per cent of the aluminum was leached out. Sweet clover growing on leached soil was better than that growing on unleached soil. The fact conclusively proved that aluminum is the determining factor in the acidity of the soils under investigation and probably of most other acid soils of the same origin.

The form of aluminum immediately concerned in the unproductivity of acid soils in the soluble form, is the salts. These salts are derived from the acid-soluble aluminum hydroxide, or gibbsite. In soils sufficiently provided with calcium, toxic aluminum salts may never be formed, but in soils deficient in calcium and other bases, as in the case of acid soils, toxic aluminum salts are largely the end-products of sulfofication and nitrification.

It is not denied that iron and manganese may become contributing factors in the unproductivity of some acid soils, but the preponderance of evidence points to aluminum as the determining factor in the acidity of the soils under investigation.

The potassium nitrate extract of an acid soil is acid, but the same extract after sufficient amounts of limestone have been applied to the soil is alkaline.

In the first case an exchange of bases takes place between the aluminum compounds and the potassium-nitrate solution bringing aluminum into solution and forming aluminum nitrate, which on hydrolysis produces strong acidity. This is the cause of the acid reaction of the solution. In the second case an exchange of bases also takes place, but this time between the calcium compounds and the potassium nitrate solution, bringing calcium into solution and forming calcium nitrate. This compound is not hydrolyzed and therefore will not produce acidity. This explains the neutral or alkaline reaction of the extract.

In so far as aluminum is a factor in soil acidity the Hopkins method is the best one for soil-acidity determinations. It determines active aluminum and under field conditions when the lime requirement of the soil has been satisfied with the amount of calcium carbonate as determined by the method, the toxicity of aluminum is eliminated.

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REFERENCES

(1) ABBOTT, J. B., CONNER, S. D., AND SMALLEY, H. R. 1913 Soil acidity, nitrification and the toxicity of soluble salts of aluminum. Ind. (Purdue Univ.) Agr. Exp. Sta. Bul. 170.

(2) AMES, J. W., AND BOLTZ, G. E. 1917 Effect of sulfofication on potassium and other soil constituents. *In* Soil Sci., v. 7, no. 3, p. 183–195.

(3) AMES, J. W., AND SCHOLLENBERGER, C. J. 1916 Liming and lime requirement of soil. Ohio Agr. Exp. Sta. Bul. 306.

(4) BAGULEY, A. 1912 The phosphate nutrition of plants. *In* Jour. Agr. Sci., v. 4, pt. 33, p. 318–322.

(5) BEMMELEN, J. M., VAN 1878 Das Absorptionwermögender Ackerde. *In* Landw. Vers. Stat., Bd. 21, p. 135.

(6) BERZELIUS, JÖNS JACOB 1856 Lehrbuch der Chemie, Bd. 3, Aufl. 8, p. 389. Leipzig.

(7) BEYER, S. W., and WILLIAMS, I. A. 1903 Technology of clays. *In* Iowa Geol. Survey Ann. Rpt. 1903, v. 14, p. 29–318.

(8) BLAIR, A. W., AND MACY, E. J. 1908 Acid soils. Fla. Agr. Exp. Sta. Bul. 93.

(9) BOULLANGER, E. 1912 Études expérimentales sur les engrais catalytiques. *In* Ann. Sci. Agron., ser. 4, t. 1, no. 3, p. 161–180.

(10) BURD, J. S. 1918 Chemical criteria, crop production and physical classification in two soil classes. *In* Soil Sci., v. 5, no. 5, p. 405.

(11) CAMERON, F. K., AND BELL, JAMES M. 1905 The mineral constituents of the soil solution. U. S. Dept. Agr. Bur. Soils Bul. 30.

(12) CLARK, F. W. 1916 The data of geochemistry. U. S. Geol. Survey Bul. 616, 3rd ed., p. 34.

(13) CONNER, S. D. 1916 Acid soils and the effect of acid phosphate and other fertilizers upon them. *In* Jour. Indus. Engin. Chem., v. 8, no. 1, p. 35.

(14) DAIKUHARA, G. 1914 Ueber saure Mineralböden. *In* Bul. Imp. Cent. Agr. Exp. Sta. Japan, v. 2, no. 1, p. 1–41.

(15) EDWARDS, M. G. 1914 The occurrence of aluminum hydrates in clays. *In* Econ. Geol., v. 9, p. 117.

(16) EICHHORN, PROF. 1877 Ueber die Einwirkung humus reicher erden auf Salze, besonder Phosphorasauren Kalk. *In* Landw. Jahrb., Bd. 1, p. 957.

(17) FAILYER, G. H., SMITH, J. G., AND WADE, H. 1908 The mineral composition of soil particles. U. S. Dept. Agr. Bur. Soils Bul. 54.

(18) FLURI, M. 1908 Der Einfluss von Aluminum salzen auf das Protoplasma. *In* Flora, Bd. 99, No. 2, p. 81–126.

(19) FREAR, W. 1915 Sour soils and liming. Com. Penn. Dept. Agr. Bul. 261.

(20) FUNCHESS, M. J. 1918 The development of soluble manganese in acid soils as influenced by certain nitrogenous fertilizers. Ala. Agr. Exp. Sta. Bul. 201 (Tech. Bul. 4).

(21) GALPIN, S. L. 1912 Studies of flint clays and their associates. *In* Trans. Amer. Ceram. Soc., v. 14, p. 301.

(22) GILLESPIE, L. J. 1916 The reaction of soil and soil measurement of hydrogen-ion concentration. *In* Proc. Wash. Acad. Sci., v. 6, no. 1, p. 7–16.

(23) GULLEY, A. 1915 Humus acids in the light of the results of recent investigation. *In* Internat. Mitt. Bodenk., Bd. 5, No. 3, p. 232–247: No. 4, p. 347–368; *Abs. in* Exp. Sta. Rec., v. 35, p. 628.

(24) HALL, A. D., AND GIMINGHAM, C. T. 1907 The interaction of ammonium salts and the constituents of the soil. *In* Jour. Chem. Soc. (London), v. 91, pt. 1, p. 677.

(25) HARRIS, J. E. 1914 Soil acidity. Mich. Agr. Exp. Sta. Tech. Bul. 19.

(26) HARRIS, J. E. 1914 Some adsorption phenomena in soils and kaolin. *In* Jour. Phys. Chem., v. 19, p. 355.

(27) HARTWELL, B. L., AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soils on barley and rye. *In* Soil Sci., v. 6, no. 4, p. 259.

(28) HARTWELL, B. L., AND PEMBER, F. R. 1919 Lime requirements as determined by the plant and by the chemist. *In* Soil Sci., v. 7, no. 4, p. 279.

(29) HEBERT, A. 1907 Toxicite des sels de chrome d'aluminum et de magnesium; leur action sur deverses fermentations. Comparaison avec les proprietes analogues des terres rares. *In* Bul. Soc. Chim. Paris, ser. 4, no. 1, p. 1026–1032.

(30) HILGARD, E. W. 1914 Soils, p. 389. Macmillan Co., New York.

(31) HILLEBRAND, W. F. 1905 The analysis of silicate and carbonate rocks. U. S. Geol. Survey Bul. 305, Ser. E.

(32) HOPKINS, C. G. 1910 Soil Fertility and Permanent Agriculture. Ginn and Co., New York.

(33) HOPKINS, C. G., KNOX, W. H., and PETTIT, J. H. 1903 A quantitative method for determining the acidity of soils. U. S. Dept. Agr. Bur. Chem. Bul. 73, p. 114.

(34) HOPKINS, C. G., AND PETTIT, J. H. 1910 Soil Fertility Laboratory Manual. Ginn and Co., New York.

(35) HOUSE, H. D., AND GIES, W. J. 1905–1906 The influence of aluminum ions on lupine seedlings. *In* Amer. Jour. Physiol., v. 15, p. 19.

(36) HUNT, T. F. 1909 Soil fertility. Penn. Agr. Exp. Sta. Bul. 90.

(37) JOHNSON, S. W. 1913 How Crops Grow. Orange Judd Co., New York.

(38) Jost, L. 1907 Plant Physiology. Translation by R. J. Harvey Gibson, p. 86. Clarendon Press, Oxford.

(39) Kratzmann, E. 1914 The physiological action of the salts of aluminum upon plants. In Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Bd. 123, II, III, p. 211–233; *Abs. in* Exp. Sta. Rec., v. 34, p. 525.

(40) Lindgren, W. 1913 Mineral Deposits, p. 326. New York.

(41) Loew, O. 1913 Studies on acid soils of Porto Rico. Porto Rico Agr. Exp. Sta. Bul. 13.

(42) Lyman, J. B., Martin, F. O., and Pearce, J. R. 1914 The centrifugal method of mechanical soil analysis. U. S. Dept. Agr. Bur. Soils Bul. 24. See also Bul. 84 of the same bureau.

(43) Maze, P. 1915 Determination des elements mineraux rares necessaires an developpement du mais. *In* Compt. Rend. Acad. Sci. (Paris), t. 160, p. 211–214.

(44) Myers, C. N. 1914 Soluble aluminum compounds—their occurrence in certain vegetable products. *In* U. S. Publ. Health Rpt., v. 29, no. 25, p. 1625–1629.

(45) Micheels, H., and DeHeen, P. 1905 Note an sujet de l'action des sels d'aluminum sur la germination. *In* Bul. Acad. Roy. Belg., (1905), p. 520.

(46) Miyake, K. 1916 The toxic action of soluble aluminum salts upon the growth of the rice plant. *In* Jour. Biol. Chem., v. 25, p. 23.

(47) Munerati, O., Mezzadroli, G., and Zapparoli, T. V. 1913 Influence of certain stimulants on the development of sugar beets. *In* Staz. Sper. Agr. Ital., v. 46, no. 7–8. p. 486–498; *Abs. in* Exp. Sta. Rec., v. 31, p. 233.

(48) Olsen, J. C. 1918 Physical constants of inorganic compounds. *In* Van Nostrand's Chemical Annual, Fourth Issue, p. 130. New York.

(49) Parker, E. G. 1914 Selective adsorption. *In* Jour. Indus. Engin. Chem., v. 6, p. 831.

(50) Pfeffer, W. 1899 Physiology of Plants. Translation by A. J. Ewart, v. 1, p. 437. Clarendon Press, Oxford.

(51) Pfeiffer, Th., and Blank. 1913 Beitrag zur Frage über die Wirkung des Mangans bezw. aluminums auf Pflanzen-wachstum. *In* Landw. Vers. Stat., Bd. 83, No. 3–4, p. 257–281.

(52) Prianischnikov, D. 1911 Uber der Einfluss von kohlen-sauren Kalk auf die Wirkung von verschiedenen Phosphaten. *In* Landw. Vers. Stat., Bd. 75, No. 5–6, p. 357.

(53) Richards, J. W. 1890 Aluminum: Its History, Occurrence, Properties, Metallurgy and Applications, Including its Alloys; 2nd ed., p. 46. Laird and Lee, Chicago.

(54) Ries, H. 1914 Clays, their Occurrence, Properties and Uses; 2nd ed., p. 202.

(55) Robinson, W. O. 1914 The inorganic composition of some important American soils. U. S. Dept. Agr. Bul. 122.

(56) Robinson, W. O., Steinkoenig, L. A., and Fry, W. H. 1917 Variation in the chemical composition of soils. U. S. Dept. Agr. Bul. 551.

(57) Robinson, W. O., Steinkoenig, L. N., and Miller, C. F. 1917 The relation of some of the rarer elements in soils and plants. U. S. Dept. Agr. Bul. 600.

(58) Rolfe, C. W. Geology of Clays. Ill. Geol. Surv. Bul. 9.

(59) Ruprecht, R. W. 1915 Toxic effect of iron and aluminum salts on clover seedlings. Mass. Agr. Exp. Sta. Bul. 161.

(60) Ruprecht. R. W., and Morse, F. W. 1915 The effect of sulfate of ammonia on soil. Mass. Agr. Exp. Sta. Bul. 165.

(61) Ruprecht, R. W., and Morse, F. W. 1917 The cause of the injurious effect of sulfate of ammonia when used as a fertilizer. Mass. Agr. Exp. Sta. Bul. 176.

(62) Sharp, L. T., and Hoagland, D. R. 1915 Acidity and adsorption in soils as measured by the hydrogen electrode. *In* Jour. Agr. Res., v. 7, p. 123–145.

(63) Shorey, E. C. 1912 Some constituents of humus. *In* Proc. Internat. Cong. Appl. Chem., v. 15, p. 247–252.

(64) SOLENOW, N. 1909 Die Boden acidität und ihre Bedeutung für den Kulturboden. Inaug. Dissert, Jena. (Original article not seen.)

(65) SPRENGER, C. 1826 Ueber Pflanzenhumus, Humussaüre und humussaure Salze. *In* Kastner Arch. Gesam. Naturl. Nürnberg, Bd. 8, p. 145–220. (Original article not seen.)

(66) STEINKOENIG, L. A. 1915 Distribution of certain constituents in the separates of loam soils. *In* Jour. Indus. Engin. Chem., v. 6, no. 7, p. 576.

(67) STODDART, C. W. 1916 Chemistry of Agricultur, p. 242. Lea and Febiger, New York.

(68) STOKLASA, J. 1911 Catalytic fertilizers for sugar beets. *In* Bl. Zuckerrühenbau, Bd. 18, No. 11, p. 193–197; *Abs. in* Exp. Sta. Rec., v. 26, p. 225.

(69) STORER, F. H. 1899 Agriculture, v. 2, p. 214–15. Scribner and Sons, New York.

(70) SULLIVAN, E. C. 1907 The interaction between minerals and water solutions. U. S. Geol. Survey Bul. 312.

(71) TACKE, BR., AND SÜCHTING, H. 1911 Ueber Humussauren. *In* Landw. Jahrb., Bd. 41, p. 717.

(72) THORNE, C. E. 1909 Carriers of phosphorus in fertilizers. Ohio Agr. Exp. Sta. Cir. 93.

(73) TREADWELL, F. P. Analytical Chemistry. Translation by Hall, W. T., 4th ed., v. 2, p. 126, 150. Wiley and Sons, New York.

(74) TRUOG, E. 1913 Availability of phosphate to various crops. *In* Wis. Agr. Exp. Sta. Bul. 240, p. 22.

(75) TRUOG, E. 1915 Soil acidity and methods for its detection. *In* Science, v. 42, p. 505.

(76) TRUOG, E. 1916 The utilization of phosphate by agricultural crops, including a new theory regarding the feeding power of plants. Wis. Agr. Exp. Sta. Res. Bul. 41.

(77) TRUOG, E. 1916 The cause and nature of soil acidity with special regard to colloids and adsorption. *In* Jour. Phys. Chem., v. 20, no. 6, p. 457.

(78) VAN SLYKE, L. L. 1919 Fertilizers and Crops, p. 112. Orange Judd Co., New York.

(79) VARVARO, U. 1912 The action of manganese dioxide and other metallic compounds on the germination of seed. *In* Staz. Sper. Agr. Ital., v. 45, no. 12, p. 917–929; *Abs. in* Exp. Sta. Rec., v. 29, p. 528.

(80) VEITCH, F. P. 1904 Comparison of methods for the estimation of soil acidity. *In* Jour. Amer. Chem. Soc., v. 10, p. 637.

(81) VOELCKER, J. A. 1909 Pot culture experiments. *In* Jour. Roy. Agr. Soc. England, v. 70, p. 394.

(82) WHEELER, H. J. 1913 Manures and Fertilizers, p. 216. Macmillan Co., New York.

(83) WHEELER, H. J., AND TOWAR, J. B. 1893 On the occasional ill-effect of sulfate of ammonia as a manure and the use of air-slacked lime in overcoming the same. *In* Ann. Rpt. R. I. Agr. Exp. Sta., v. 6, p. 206.

(84) YAMANO, G. 1905 Can aluminum salts enhance plant growth? *In* Bul. Col. Agr., Tokyo Imp. Univ., v. 6, no. 4, p. 429–432.

PLATE 1

EFFECT OF ALUMINUM SULFATE ON THE GROWTH OF SWEET CLOVER

FIG. 1. First crop, 93 days old.

101—Control—plant-food only.

102—Plant-food plus 3100 pounds of $Al_2(SO_4)_3$ per acre.

103—Plant-food plus 620 pounds of $Al_2(SO_4)_3$ per acre.

104—Plant-food plus 15,500 pounds of $Al_2(SO_4)_3$ per acre.

105—Plant-food plus 3100 pounds of $Al_2(SO_4)_3$ per acre plus 2716 pounds of $CaCO_3$.

106—Plant-food plus 3100 pounds of $Al_2(SO_4)_3$ per acre plus 543 pounds of $CaCO_3$.

107—Plant-food plus 3100 pounds of $Al_2(SO_4)_3$ plus 13,580 pounds of $CaCO_3$.

108—Plant-food plus 3100 pounds of $Al_2(SO_4)_3$ per acre plus 1054 pounds of $CaCO_3$.

109—Plant-food plus 3100 pounds of $Al_2(SO_4)_3$ per acre plus 211 pounds of $CaCO_3$.

110—Plant-food plus 3100 pounds of $Al_2(SO_4)_3$ per acre plus 5270 pounds of $CaCO_3$.

FIG. 2. Second crop, 96 days old. The same as above plus $CaSiO_3$ and acid phosphate, reduced from 100 to 400 pounds per acre.

194

JOSE JISON MIRASOL
PLATE 1

FIG. 1

FIG. 2

195
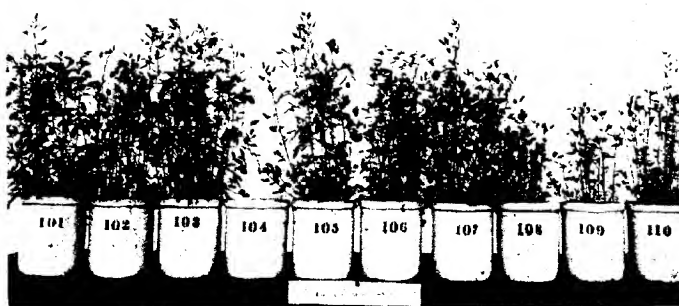
EFFECT OF ALUMINUM CHLORIDE ON SWEET CLOVER

FIG. 1. Sweet clover at the age of 44 days.

201—Control—Plant-food only.

202—Plant-food plus 2405 pounds of AlCl₃ per acre.

203—Plant-food plus 461 pounds of AlCl₃ per acre.

204—Plant-food plus 12,025 pounds of AlCl₃ per acre.

205—Plant-food plus 2405 pounds of AlCl₃ plus 2716 pounds of CaCO₃ per acre.

206—Plant-food plus 2405 pounds of AlCl₃ plus 543 pounds of CaCO₃ per acre.

207—Plant-food plus 2405 pounds of AlCl₃ plus 13,580 pounds of CaCO₃ per acre.

208—Plant-food plus 2405 pounds of AlCl₃ plus 1054 pounds of CaH₄(PO₄)₂ per acre.

209—Plant-food plus 2405 pounds of AlCl₃ plus 211 pounds of CaH₄(PO₄)₂ per acre.

210—Plant-food plus 2405 pounds of AlCl₃ plus 5270 pounds of CaH₄(PO₄)₂ per acre.

FIG. 2. Same, at the age of 93 days. Treatment, same as above.

FIG. 1

FIG. 2

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PLATE 3

EFFECT OF ALUMINUM NITRATE ON SWEET CLOVER

FIG. 1. First crop, 93 days old.

301—Control—Plant-food only.

302—Plant-food plus 3859 pounds of $Al(NO_3)_3.9H_2O$ per acre.

303—Plant-food plus 752 pounds of $Al(NO_3)_3.9H_2O$ per acre.

304—Plant-food plus 19,295 pounds of $Al(NO_3)_3.9H_2O$ per acre.

305—Plant-food plus 3859 pounds of $Al(NO_3)_3.9H_2O$ plus 2716 pounds $CaCO_3$ per acre.

306—Plant-food plus 3859 pounds of $Al(NO_3)_3.9H_2O$ plus 543 pounds of $CaCO_3$ per acre.

307—Plant-food plus 3859 pounds of $Al(NO_3)_3.9H_2O$ plus 13,580 pounds of $CaCO_3$ per acre.

308—Plant-food plus 3859 pounds of $Al(NO_3)_3.9H_2O$ plus 1054 pounds of $CaH_4(PO_4)_2$ per acre.

309—Plant-food plus 3859 pounds of $Al(NO_3)_3.9H_2O$ plus 211 pounds of $CaH_4(PO_4)_2$ per acre.

310—Plant-food plus 3859 pounds of $Al(NO_3)_3.9H_2O$ plus 5270 pounds of $CaH_4(PO_4)_2$ per acre.

FIG. 2. Second crop, 96 days old. Same as above with the same changes as noted in series 100.

Fig. 1
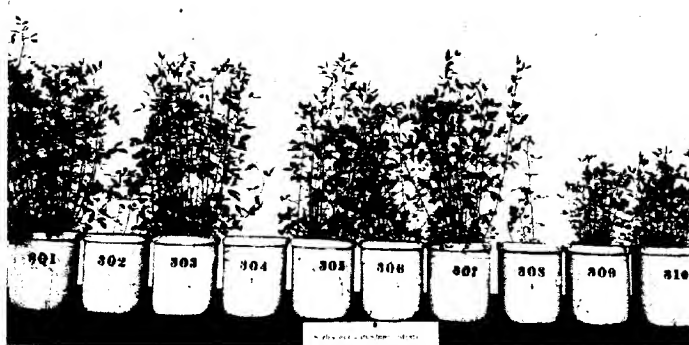
Fig. 2

PLATE 4

EFFECT OF ALUMINUM HYDROXIDE ON SWEET CLOVER

FIG. 1. First crop, 93 days old.

401—Control—Plant-food only.

402—Plant-food plus 1399 pounds of $Al(OH)_3$ per acre.

403—Plant-food plus 280 pounds of $Al(OH)_3$ per acre.

404- Plant-food plus 6995 pounds of $Al(OH)_3$ per acre.

405—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 2716 pounds of $CaCO_3$ per acre.

406—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 543 pounds of $CaCO_3$ per acre.

407—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 13,580 pounds of $CaCO_3$ per acre.

408—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 1054 pounds of $CaH_4(PO_4)_2$ per acre.

409—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 211 pounds of $CaH_4(PO_4)_2$ per acre.

410—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 5270 pounds of $CaH_4(PO_4)_2$ per acre.

FIG. 2-3. Second crop, 96 days old. Same as above up to 410 with the same changes as noted in series 100 to 300.

411—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 800 pounds of $(NH_4)_2SO_4$ per acre.

412 -Plant-food plus 1399 pounds of $Al(OH)_3$ plus 160 pounds of $(NH_4)_2SO_4$ per acre.

413—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 4000 pounds of $(NH_4)_2SO_4$ per acre.

414—Plant-food plus 6995 pounds of $Al(OH)_3$ plus 800 pounds of $(NH_4)_2SO_4$ per acre.

415—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 800 pounds of $(NH_4)_2SO_4$ plus 2716 pounds of $CaCO_3$ per acre.

416—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 800 pounds of $(NH_4)_2SO_4$ plus 543 pounds of $CaCO_3$ per acre.

417 -Plant-food plus 1399 pounds of $Al(OH)_3$ plus 800 pounds of $(NH_4)_2SO_4$ plus 13,580 pounds of $CaCO_3$ per acre.

418—Plant-food plus 1399 pounds of $Al(OH)_3$ plus 2423 pounds of dried blood per acre.

419 -Plant-food plus 1399 pounds of $Al(OH)_3$ plus 484 pounds of dried blood per acre.
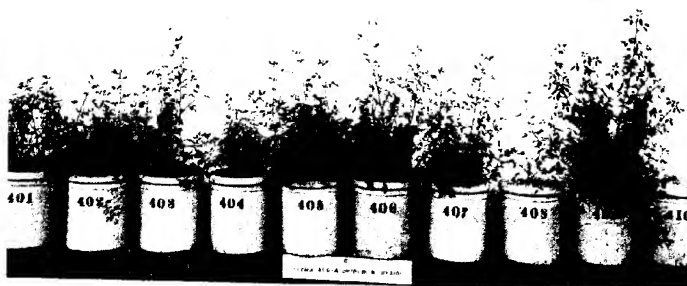
Fig. 1
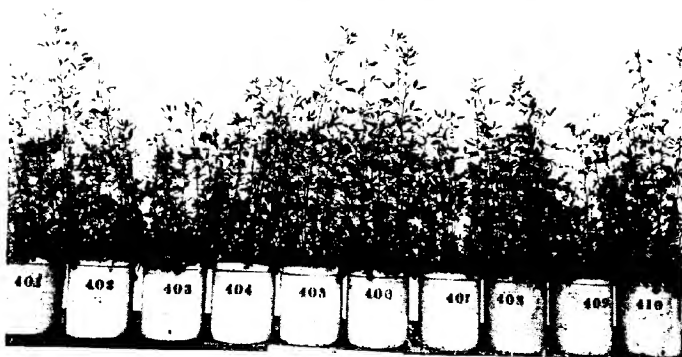
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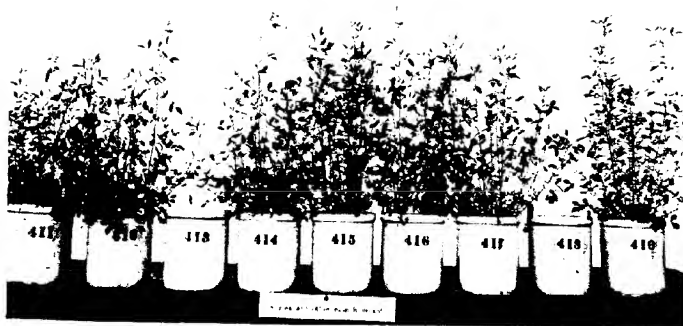
Fig. 3

PLATE 5

EFFECT OF ACID PHOSPHATE ON SWEET CLOVER

FIG. 1. First crop, 75 days old.

601—Control—Plant-food only.

602—Plant-food plus 964 pounds of $CaH_4(PO_4)_3$ per acre.

603—Plant-food plus 193 pounds of $CaH_4(PO_4)_3$ per acre.

604—Plant-food plus 4820 pounds of $CaH_4(PO_4)_3$ per acre.

605—Plant-food plus 964 pounds of $CaH_4(PO_4)_3$ plus 2921 pounds of $CaCO_3$ per acre.

606—Plant-food plus 964 pounds of $CaH_4(PO_4)_3$ plus 584 pounds of $CaCO_3$ per acre.

607—Plant-food plus 964 pounds of $CaH_4(PO_4)_3$ plus 14605 pounds of $CaCO_3$ per acre.

FIG. 2. Second crop, 67 days old. Amounts of acid phosphate reduced to from 100 to 400 pounds per acre.

FIG. 1

FIG. 2

PLATE 6
JOSE JISON MIRASOL

FIG. 1

FIG. 2

EFFECT OF LIMESTONE AND ACID PHOSPHATE ON THE PRODUCTIVITY OF YELLOW GRAY SILT LOAM

FIG. 1. First crop, 98 days old.

801—Control.

802—2813 pounds of limestone per acre.

803—562 pounds of limestone per acre.

804—14,065 pounds of limestone per acre.

805—3080 pounds of $CaH_4(PO_4)_2$ per acre.

806—616 pounds of $CaH_4(PO_4)_2$ per acre.

807—15,400 pounds of $CaH_4(PO_4)_2$ per acre.

808—3080 pounds of $CaH_4(PO_4)_2$ plus 2813 pounds of limestone per acre.

809—3080 pounds of $CaH_4(PO_4)_2$ plus 562 pounds of limestone per acre.

810—3080 pounds of $CaH_4(PO_4)_2$ plus 14,065 pounds of limestone per acre.

FIG. 2. Second crop, 123 days old. Treatment, same as above

FIG. 1

FIG. 2

FIG. 1

FIG. 2

PLATE 9

A. Water-leached soil.
A-1. Potassium-nitrate-leached soil.
A-2. Original soil plus KNO_3 equal to excess of KNO_3 in A-1.
A-3. Original soil.

Potassium nitrate-leached soil

PLATE 10

GRAPHS SHOWING THE DECREASE OF ACIDITY OF SOIL DUE TO TREATMENT — GRAY
SILT LOAM

Curve 1 represents the per cent decrease of acidity in 108 days; curve 2 the per cent decrease in 178 days.

701—Control

702—2125 pounds of limestone per acre.

703- 425 pounds of limestone per acre.

704—10,625 pounds of limestone per acre.

705—2310 pounds of $CaH_4(PO_4)_2$ per acre.

706—462 pounds of $CaH_4(PO_4)_2$ per acre.

707- -11,550 pounds of $CaH_4(PO_4)_2$ per acre.

708—2310 pounds of $CaH_4(PO_4)_2$ plus 2125 pounds of limestone per acre.

709—2310 pounds of $CaH_4(PO_4)_2$ plus 425 pounds of limestone per acre.

710—2310 pounds of $CaH_4(PO_4)_2$ plus 10,625 pounds of limestone per acre.
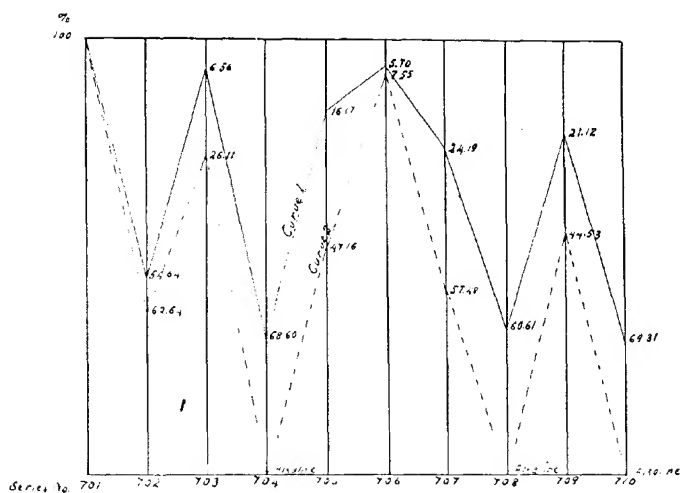
PLATE 11

GRAPHS SHOWING THE DECREASE OF ACIDITY OF SOIL DUE TO TREATMENT—YELLOW
GRAY SILT LOAM

Curve 1 represents the per cent decrease of acidity in 108 days; curve 2 the per cent decrease of acidity in 178 days.

801—Control.

802—2813 pounds of limestone per acre.

803—562 pounds of limestone per acre.

804—14,065 pounds of limestone per acre.

805—3080 pounds of $CaH_4(PO_4)_2$ per acre.

806- 616 pounds of $CaH_4(PO_4)_2$ per acre.

807—15,400 pounds of $CaH_4(PO_4)_2$ per acre.

808— 3080 pounds of $CaH_4(PO_4)_2$ plus 2813 pounds of limestone per acre.

809 —3080 pounds of $CaH_4(PO_4)_2$ plus 562 pounds of limestone per acre.

810 —3080 pounds of $CaH_4(PO_4)_2$ plus 14,065 pounds of limestone per acre.
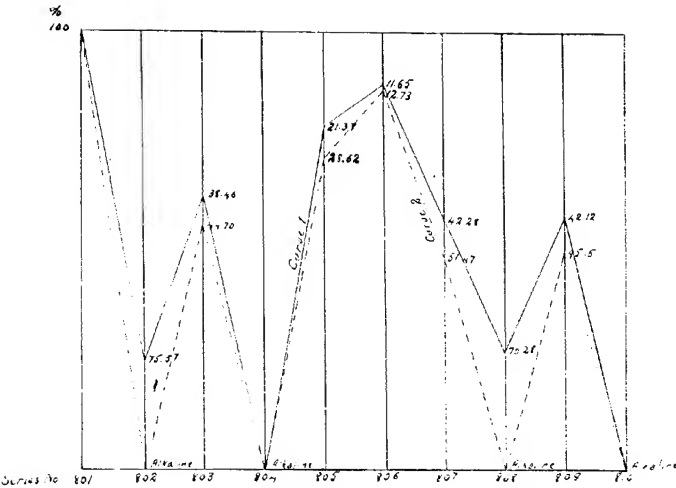
GRAPHS SHOWING THE DECREASE OF ACIDITY OF SOIL DUE TO TREATMENT—YELLOW
SILT LOAM

Curve 1 represents the per cent decrease of acidity in 108 days; curve 2 the per cent decrease of acidity in 178 days.

901—Control.

902—2921 pounds of limestone per acre.

903—584 pounds of limestone per acre.

904—14.605 pounds of limestone per acre.

905   3080 pounds of $CaH_4(PO_4)_2$ per acre.

906—616 pounds of $CaH_4(PO_4)_2$ per acre.

907—15.400 pounds of $CaH_4(PO_4)_2$ per acre.

908—3080 pounds of $CaH_4(PO_4)_2$ plus 2921 pounds of limestone per acre.

909—3080 pounds of $CaH_4(PO_4)_2$ plus 594 pounds of limestone per acre.

910—3080 pounds of $CaH_4(PO_4)_2$ plus 14,605 pounds of limestone per acre.

217
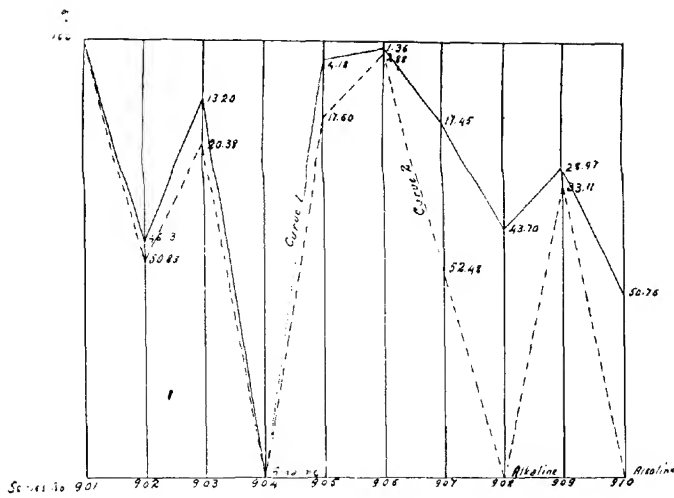
THE FORMATION OF SOLUBLE SUBSTANCES IN SOILS TAKEN FROM WIDELY SEPARATED REGIONS

M. M. McCool and C. E. Millar

Michigan Agricultural Experiment Station

The rate of formation of soluble substances in soils has received a great deal of study by various members of the Soils Section. The freezing-point method, perfected by Bouyoucos (1), in the main has been utilized for these investigations, yet the composition of the material going into solution has received attention (7). Our plans call for extensive researches along these lines. Millar is investigating the composition of the soil solution of cropped and virgin soils, while Spurway (9) and Wheeting, respectively, are considering the residuary effects of salts on soils of different texture.

In discussing with Prof. C. F. Marbut, of the United States Bureau of Soils, the origin, weathering, composition and other questions that arise in connection with the soil survey work, it occurred to us that an investigation of the relative rate of formation of soluble salts in soils derived from material of different nature and formed under various climatic conditions as well as in soils which might be classed as very old, intermediate, and young, would be interesting and profitable. Accordingly through the courtesy of Professor Marbut and several members of the corps of field men engaged in soil survey work, a number of such samples were obtained. Many samples were supplied also by the men in charge of the soils work and outlying experimental stations of several states. Samples were received from the following states: Texas, Ohio, California, Washington, Arizona, Kentucky, Nevada, Tennessee, Michigan, Kansas, Indiana, Missouri, Georgia and Alabama. A brief description of the soil and subsoil follows the name used to characterize each sample. The numbers correspond to those on the map and those preceding the soil names in the tables of data. Unless otherwise indicated the surface samples were taken to a depth of 8 inches and the subsoils from 24 to 36 inches. The regions from which the samples were obtained are shown on the accompanying sketch map.

DESCRIPTION OF SOILS

1. Cochise, Arizona. This is a chocolate-brown clayey sand containing much coarse material. The subsoil is a calcareous red clay intermixed with much coarse material. This sample is from the Sulfur Springs Valley dry farm which has an elevation of about 4,000 feet and an annual precipitation of approximately 11 inches.

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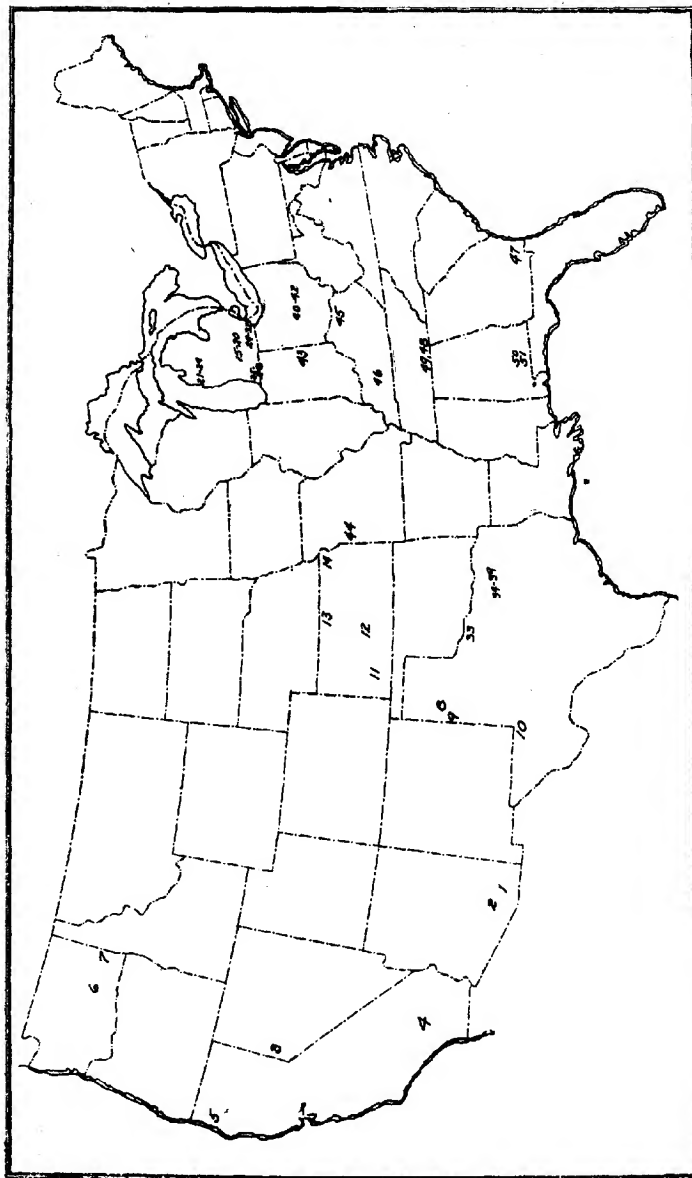
FIG. 1. MAP SHOWING REGIONS FROM WHICH SOIL SAMPLES WERE OBTAINED

2. Tucson, Arizona. The soil consists of calcareous gray fine sand. The subsoil is of similar material. This soil represents a virgin area in the Rilleto River bottom on the University Farm. The rainfall in this locality is between 11 and 12 inches and the elevation is about 2300 feet.

3. Reno, Nevada. This is a brown sandy loam with a brown sandy loam subsoil. This sample was taken from the Experiment Station farm which is under irrigation.

4. Whittier, California. This is a clay loam with a dark gray clay subsoil and was derived from shale, sandstone and other metamorphic rocks. Depth of sample: surface 12 inches; subsoil 24 inches.

5. Riverside, California. Classified as Placentia light sandy loam with a coarse brown sandy loam subsoil. This soil is of granitic origin. Depth of sample: surface 12 inches; subsoil 12 to 24 inches.

6. Lind, Washington. This is a very fine sand with a yellow very fine sandy loam subsoil.

7. Pullman, Washington. This soil is a dark silt loam with a yellow clay subsoil.

Texas soils

8. Spur. This is a chocolate-brown clay with a reddish brown clay subsoil. The sample was taken from a virgin area on the experiment station farm.

9. Lubbock. 1. This soil is classified by the Bureau of Soils as Armarillo fine sandy loam. The subsoil is a calcareous brown fine sandy loam underlaid with marl at from 3 to 4 feet.

9a. Lubbock. 2. A heavy fine sandy loam classified by the Bureau of Soils as Richfield loam. The subsoil is a calcareous heavy sandy loam underlaid with marl at from 2 to 4 feet.

10. Pecos. This is a gray light sandy loam with a gray sandy clay subsoil. The sample represents a virgin area on the Experiment Station farm.

Kansas soils

11. Richland silt loam. The sample was taken from an irrigated field in Finney County. It has grown grain crops for 20 years. Depth of sample: surface 7 inches.

12. Arkansas fine sandy loam. This soil represents the bottom land along the Arkansas River. The sample was taken from a field in Pawnee County which has been cropped for 20 years. The water-table comes to within about 4 feet of the surface. Depth of sample: surface 7 inches.

13. Lincoln silt loam. This soil came from Jewell County and is considered the best general farming soil in the county. The sample came from a field which has grown corn and wheat for 30 years. Depth of sample: surface 7 inches.

14. Marshall silt loam. This sample came from a field in Brown County, which has been cropped to corn, wheat and oats for 45 years. Depth of sample: surface 7 inches.

Michigan soils

15. Miami silt loam. A brown silt loam with a brown clay loam subsoil. This soil has been cropped many years with little effort to maintain its productivity.

16. Miami silt loam. Similar to the above but from an uncropped area.

17. Miami very fine sandy loam. A brownish gray soil with a clayey sand subsoil. Representative of a very productive type of central Michigan soil.

18. Clyde sandy loam. A dark gray soil with gray sandy clay subsoil.

19. Clyde silt loam. A very heavy gray silt loam with clay loam subsoil. Representative of small areas in central Michigan.

20. Miami sandy loam. A rather heavy brown sandy loam with clayey sand subsoil. Representative of much of the general farming land in central Michigan.

21. Coloma sand. Level to undulating land originally covered with pine timber. Subsoil quite sandy.

22. Plainfield sand. Terrace along the Manistee River. Subsoil medium sand.

23. Miami silt loam. Rolling brown silt loam underlaid with yellow, mottled clay loam.

24. Coloma sand. Brown sand underlaid with yellow sand. Original timber pine.

25. Silt loam. Rolling land formerly bearing beech and maple timber. Taken from a field quite low in organic matter. Subsoil yellow sand.

26. Plainfield sand. Terrace formation along Dowagiac creek. Subsoil gray sand.

27. Coloma sand. Rolling land originally timbered with oak. Subsoil sandy. Taken from a depleted field.

28. Miami silt loam. Rolling beech and maple land. Subsoil brown clay loam.

29. Miami silt loam. Taken from a comparatively level area which is somewhat in need of drainage. This soil has been cropped quite heavily but is still productive. Subsoil is a clay loam.

30. Clyde silt loam. Representative of the black silt loams of the old lake bed area of southeastern Michigan. Badly in need of drainage. Subsoil is clay loam.

31. Miami silt loam. Area quite rolling. Representative of morainic formations in northwestern Lenawee County. Subsoil clay.

32. Coloma sand. Rolling sand, subject to blowing if not properly managed. Taken from a much depleted field. Subsoil, yellow sand.

Texas soils

33. Chillicothe. This is a deep sandy loam with a coarse clayey sand subsoil. The sample was taken from a virgin area on the Experiment Station farm and represents the average soil on the farm.

34. Ellis clay. Yellowish brown clay underlaid with yellow clay containing some bluish-gray mottling. This sample was taken from uncultivated prairie growing mesquite grass. Depth of sampling unknown.

35. Bell clay. Black clay to the depth of 12 inches. Taken from terrace land about 10 feet above high water. A very productive soil. Subsoil is a dark bluish gray clay. Gravel is found at a depth of several feet. Depth of sampling unknown.·

36. Trinity clay. Dark brown clay to the depth of 12 inches, representing an alluvial formation along the Trinity River. It is very productive. Subsoil is brown clay for many feet. Depth of sampling unknown.

37. Houston clay. A black clay 12 inches deep representing the main type of central Texas prairie. Subsoil to 36 inches is black clay containing fine particles of limestone (chalk). The original chalk material is found at a depth of 5 to 10 feet. Depth of sampling unknown.

38. Cahaba fine sandy loam. Brown loamy fine sand to a depth of 14 inches of terrace formation. Lower portion of surface soil is reddish in color. The subsoil to a depth of 36 inches is a dull brownish red friable clay. Depth of sampling unknown.

Ohio soils

40. Clyde silty clay loam. Subsoil is a mottled, drab clay.

41. Ellsworth silt loam. Subsoil is yellow clay. This soil was derived from the weathering of sandstone and shale.

42. Miami silty clay loam. Subsoil consists of yellow clay. This sample is representative of the light-colored upland soil occurring over a large part of western Ohio.

43. Miami silt loam from Delaware County, Indiana. Depth of sampling unknown.

44. Summit silt loam from Cass County, Missouri. Depth of sampling unknown.

45. Lexington, Kentucky. This soil is a brown silt loam with a yellow clay subsoil. It was taken from a virgin woodland pasture.

46. Greenville, Kentucky. The surface and subsoil of this sample both consist of yellow clay. Depth of sample: surface 8 inches; subsoil 12 to 24 inches.

47. Norfolk fine sandy loam. From Pierce County, Georgia. Surface sample taken to a depth of 4 inches. From 4 to 24 inches there is a stratum of yellowish gray fine sand. Below this depth the sand is mixed with more plastic material.

48. Lebanon (Clarksville) silt loam from Tennessee. This sample was taken near Tullahoma, Tennessee, from a forest of blackjack oak, post oak, red oak and black hickory. Depth of sample 8 inches—subsoil taken from 8 to 25 inches.

49. Hagerstown silt loam from Tennessee. This sample was taken near Shelbyville, Tenn., and grew the following varieties of timber, red oak, post oak, white oak, hard maple, walnut and cedar. Depth of sample 8 inches.

50. Ruston fine sandy loam. Sample taken near Luverne in Crenshaw County, Alabama. Depth of sample 10 inches, subsoil 10 to 36 inches. From 10 to 24 inches the material is a dull red to yellowish red fine sandy clay. From 24 to 36 inches the material is the same but the color mottled.

51. Orangeburg fine sandy loam. The sample was collected near Luverne, Alabama, and consists of a gray fine sandy loam merging into a light reddish color in the lower depth. Depth of sample 10 inches; subsoil 10 to 36 inches. Consists of rather compact red fine sandy clay.

EXPERIMENTAL

The investigations may be divided into three phases: first, the formation of soluble material in surface and sub-soils from regions of radically different climatic conditions; second, the formation of soluble material in the various soil separates composing the soils from different regions, also the effect of grinding the coarser separates on the formation of soluble products; third, the effect of treatment with a solution of sodium nitrate on the formation of water-soluble material in the various separates obtained from certain soils.

Formation of soluble material in surface and subsoils

The samples of air-dry soil were passed through a 1-mm. screen. About 50 gm. was then placed on filter paper in a funnel and washed with distilled water until the freezing-point depression of the soil solution in the soil was practically zero. The soil was then thoroughly mixed by placing in a tumbler and stirring, and portions placed in freezing-point tubes which were stoppered and placed in a chamber maintained at 25°C. Except in the case of very heavy soils this procedure afforded sufficient moisture so that a short column of water rose above the sample in the tube, and when this was not the case sufficient water was added to bring about the above condition. At intervals the tubes were unstoppered and the contents shaken to remove any accumulated gases and provide thorough aeration. The freezing-point lowerings were determined in the usual manner after 5, 10, 30 and 60 days, unless otherwise indicated in the tables.

Table 1 shows the results for surface soils formed from various materials and under different climatic conditions. The average annual precipitation of the regions from which the samples were collected also is shown.

TABLE 1

Rate of formation of soluble material in surface* soils derived from different material and from areas of various precipitation when maintained at 25°C.

SOILS	ANNUAL PRECIPITA- TION	FREEZING-POINT DEPRESSION				
		0 days	5 days	10 days	30 days	60 days
	inches	°C.	°C.	°C.	°C.	°C.
Arizona soils						
1. Cochise....................	11.00	0.000	0.003	0.010	0.010	0.008
2. Tucson.....................	12.00	0.000	0.009	0.010	0.017	0.016
Nevada soils						
3. Reno......................	8.30†	0.000	0.002	0.012	0.014	0.027
California soils						
4. Whittier‡.................	13.00	0.000	0.010	0.025	0.027	0.035
5. Riverside‡................	10.00	0.000	0.002	0.004	0.005	
Washington soils						
6. Lind.....................	9.50	0.000	0.007	0.004	0.008	0.015
7. Pullman...................	21.64	0.000	0.014	0.010	0.019	0.034
Texas soils						
8. Spur......................	21.00	0.000	0.028		0.027	0.026
9. Lubbock 1.................	21.00	0.000	0.010	0.020	0.029	0.024
9a. Lubbock 2.................	21.00†	0.000	0.015	0.026	0.029	0.028
10. Pecos‡....................	21.00	0.002	0.015	0.022	0.025	0.030
		0 days	5 days	10 days	20 days	40 days
Kansas soils						
11. Richland silt loam..........	20.00†	0.003	0.030	0.039	0.031	0.010
12. Arkansas fine sandy loam....	22.90	0.001	0.022	0.034	0.043	0.010
13. Lincoln silt loam...........	26.30	0.000	0.020	0.031	0.032	0.012
14. Marshall silt loam..........	33.35	0.002	0.010	0.012	0.020	0.001
Michigan soils						
Ingham County						
15. Miami silt loam............	30.99	0.000	0.009	0.012	0.012	0.018
16. Miami silt loam............	30.99	0.000	0.021	0.034	0.045	0.035
17. Miami very fine sandy loam.	30.99	0.000	0.008	0.010	0.019	0.024
18. Clyde sandy loam...........	30.99	0.001	0.013	0.034	0.030	0.030
19. Clyde silt loam............	30.99	0.000	0.016	0.032	0.026	0.029
20. Miami sandy loam..........	30.99	0.006	0.015	0.019	0.030	0.035
Manistee County						
21. Coloma sand....	30.27	0.000	0.002	0.011	0.024	0.020
22. Plainfield sand.............	30.27	0.000	0.003	0.004	0.016	0.019
23. Miami silt loam............	30.27	0.000	0.028	0.038	0.035	0.039
24. Coloma sand..............	30.27	0.000	0.011	0.013	0.011	0.010
Berrien County						
25. Silt loam...................	34.15	0.001	0.012	0.013	0.013	0.026
26. Plainfield medium sand......	34.15	0.000	0.009	0.011	0.012	0.022
27. Coloma sand...............	34.15	0.000	0.001	0.002	0.002	0.003
28. Miami silt loam............	34.15	0.000	0.013	0.026	0.029	0.038

TABLE 1—*Continued*

SOILS	ANNUAL PRECIPITA-TION	FREEZING-POINT DEPRESSION				
		0 days	5 days	10 days	20 days	40 days
	inches	°C.	°C	°C.	°C.	°C.
Lenawee County						
29. Miami silt loam.............	37.03	0.000	0.003	0.022	0.036	0.034
30. Clyde silt loam.............	37.03	0.000	0.014	0.021	0.031	0.016
31. Miami silt loam.............	37.03	0.000	0.017	0.037	0.038	0.038
32. Coloma sand...............	37.03	0.000	0.005	0.008	0.017	0.012

SOILS	ANNUAL PRECIPITA-TION	0 days	5 days	10 days	30 days	60 days
Texas soils						
33. Chillicothe.................	26.29	0.000	0.008	0.023	0.033	0.017
34. Ellis clay,‡ Dallas County ...	36.00	0.000	0.023	0.029	0.046	0.049
35. Bell clay,‡ Dallas County	36.00	0.000	0.010	0.020	0.030	0.032
36. Trinity clay,‡ Dallas County.	36.00	0.005	0.011	0.014	0.023	0.022
37. Houston clay,‡ Dallas County	36.00	0.000	0.011	0.014	0.022	0.020
38. Cohaba fine sandy loam,‡ Dallas County...............	36.00	0.000	0.003	0.005	0.006	0.008
Ohio soils						
40. Clyde silty clay loam........	38.80	0.003	0.033	0.028	0.013	0.020
41. Ellsworth silt loam..........	38.80	0.001	0.006	0.007	0.027	0.027
42. Miami silty clay loam	38.80	0.000	0.021	0.037	0.052	0.040
Indiana soils§						
43. Miami silt loam............	38.00	0.006	0.018	0.020	0.023	0.028
Missouri soils§						
44. Summit silt loam............	38.00	0.006	0.015	0.020	0.030	0.034
Kentucky soils						
45. Lexington................	44.80	0.000	0.015	0.016	0.016	0.020
46. Greenville.................	45.80	0.006	0.009	0.015	0.033	
Georgia soils						
47. Norfolk fine sandy loam‡....	50.00	0.009	0.012	0.012	0.016	0.015

* Samples taken to depth of 8 inches unless otherwise stated.
† Soil under irrigation.
‡ See description for depth of sampling.
§ Maintained at room temperature.

There are some very interesting and indeed fundamental indications in these results. It has long been considered that soils under arid or semi-arid conditions contain more readily soluble material than soils from other regions. While, as shown by various investigators, the amount of water-soluble salts in such soils is often quite high, the data presented show that after this material is removed by washing, the rate of formation of soluble products is comparatively slow.

If the soils are grouped with respect to the precipitation to which they are subjected and an average freezing-point depression found for each group, taking into consideration the 5 and 10-day periods only, the data of table 2 are obtained.

Since some of the groups contain only a small number of soils and some contain a number of sands which appear to be less reactive than the heavier classes, too much significance cannot be attached to these averages. However, the data are quite suggestive in that soils from regions of quite low rainfall and from regions of excessive precipitation appear to have a lower rate of solubility than those from regions of intermediate precipitation.

TABLE 2

Comparative rate of solubility of soils from regions of different precipitation when maintained at 25°C.

ANNUAL PRECIPITATION	FREEZING-POINT DEPRESSION	
	5 days	10 days
	°C.	°C.
Below 20 inches....................................	0.0067	0.0107
20 to 30 inches....................................	0.0200	0.0287
30 to 35 inches....................................	0.0114	0.0180
35 to 40 inches....................................	0.0132	0.0200
40 to 50 inches....................................	0.0120	0.0143

If a comparison is made between the soils derived from glacial material, the soils from the far west and the soils south of the glacial belt, the data of table 3 are obtained.

TABLE 3

Comparative rate of solubility of soils from the glaciated, western and southern portions of the United States, when mainatined at 25°C.

	FREEZING-POINT DEPRESSION	
	5 days	10 days
	°C.	°C.
Western soils.......................................	0.0067	0.0107
Glacial soils.......................................	0.0129	0.0200
Southern soils......................................	0.0148	0.0206

These results are interesting in that they show the western soils, or those chiefly formed by the processes of disintegration rather than of decomposition, are much slower in their rate of formation of soluble material than soils from the glaciated portions of the United States. This is somewhat at variance with earlier teachings.

It is also interesting to note that the glacial soils have practically the same rate of solubility as the soils from the unglaciated areas. This is also contrary to the generally accepted idea. There may be some doubt as to whether the soils from Kansas receiving approximately 30 inches of precipitation and those from Texas receiving a rainfall of only 21 inches should be put in the same group with the other southern soils which are subject to high precipita-

tion.   Should these soils be omitted the results for the 5-day period become 13.9 instead of 14.8, and for the 10-day period 19.8 in place of 20.0.   The inclusion of the data from these soils therefore makes no appreciable difference in the ultimate results.

Subsoils

The rate of formation of soluble material in a number of subsoils was studied in the same manner as described for the surface soils.   The data obtained, together with the annual precipitation to which the soils are subjected, are presented in table 4.

TABLE 4

Rate of formation of soluble material in subsoils* from different regions of the United States, when maintained at 25°C.

	ANNUAL PRECIPITA-TION	FREEZING-POINT DEPRESSION						
		0 days	5 days	10 days	30 days	60 days		
		°C.	°C.	°C.	°C.	°C.	°C.	°C.
1. Cochise, Arizona........	11.00	0.000	0.004	0.004	0.006	0.006		
2. Tucson, Arizona........	12.00	0.008	0.030	0.028	0.035	0.043		
3. Nevada Agricultural College..............	8.30	0.000	0.008	0.008	0.010			
4. Whittier, California, clay loam 1 to 2 ft........	13.00	0.001	0.002	0.003	0.007			
5. Riverside, California sandy loam 1 to 2 ft...	10.00	0.000	0.003	0.002	0.003			
6. Lind, Washington.......	9.50	0.000	0.008	0.007	0.007	0.007		
7. Pullman, Washington....	21.64	0.000	0.003	0.007	0.004	0.000		
8. Spur, Texas............	21.00	0.000	0.007	0.024	0.017	0.012		
9. Lubbock, Texas.........	21.00	0.000	0.007	0.008	0.015	0.010		
9a. Lubbock, Texas, sub- soil 2................	21.00	0.000	0.006	0.018	0.017	0.015		
33. Chillicothe, Texas.......	26.29	0.000	0.003	0.006	0.003	0.002		
Franklin County, Ohio								
40. Clyde silty clay loam	38.00	0.000	0.002	0.002	0.006	0.004		
41. Ellsworth silt loam..	38.80	0.000	0.005	0.000	0.009	0.005		
42. Miami silty clay loam................	38.80	0.000	0.008	0.009	0.011	0.017		
45. Lexington, Ken- tucky, 12 to 24 inches.	44.80	0.000	0.005	0.005	0.006	0.002		
46. Greensville, Ken- tucky, 12 to 24 inches.	45.80	0.000	0.001	0.004	0.003			
		0 days	1 day	4 days	10 days	20 days	40 days	60 days
Michigan soils								
15. Miami silt loam....	30.99	0.003	0.006	0.008	0.010	0.012	0.012	0.012
16. Miami silt loam....	30.99	0.000	0.001	0.006	0.004	0.004	0.000	0.000
17. Miami very fine sandy loam......	30.99	0.000	0.002	0.009	0.009	0.017	0.018	0.017
19. Clyde silt loam.....	30.99	0.000	0.005	0.011	0.011	0.012	0.008	0.007
20. Miami sandy loam..	30.99	0.000	0.002	0.001	0.002	0.000	0.000	0.000

* Depth of sampling from 2 to 3 feet unless otherwise stated.

These data show that the rate of formation of soluble substances is quite slow in practically all subsoils regardless of the material from which they were derived or the climatic conditions to which they have been subjected. The only notable exception to this observation is the sample from Tucson, Arizona, and since this is a river-bottom soil the abnormal behavior is not surprising.

This lack of solubility of subsoils is of interest in the discussion of the question as to how much of their mineral plant-food elements plants draw from the lower strata of the soil. It also opens the question as to whether the greater solubility of material in the surface soil is due to the presence of organic matter or to a more advanced condition of decay of the mineral constituents.

Solubility of very old soils

In the classification of soils Professor Marbut lays much emphasis on age. There are in the United States certain areas of soil which are recognized as extremely old, that is, from the standpoint of weathering, and in the present study it was considered very desirable to include some such samples. The specimens used were furnished by the Bureau of Soils. The procedure was the same as described above.

TABLE 5

Rate of formation of soluble material in soils which have undergone extreme weathering, maintained at 25°C.

	FREEZING-POINT DEPRESSION					
	0 days	1 day	5 days	10 days	30 days	60 days
	°C.	°C.	°C.	°C.	°C.	°C.
48. Tennessee soils						
Lebanon silt loam						
Surface 8 inches....................	0.000	0.002	0.004	0.004	0.007	0.010
Subsoil 8 to 25 inches..............	0.000	0.001	0.003	0.002	0.003	0.002
49. Hagerstown silt loam 8 inches...........	0.000	0.003	0.008	0.010	0.009	0.011
50. Alabama soils						
Rushton fine sandy loam						
Surface 10 inches...................	0.000	0.000	0.000	0.004	0.004	0.000
Subsoil 10 to 36 inches.............	0.000	0.000	0.000	0.002	0.002	0.002
51. Orangeburg fine sandy loam 10 inches.....	0.000	0.000	0.000	0.000	0.000	0.000
Subsoil 10 to 36 inches..............	0.000	0.000	0.000	0.000	0.000	0.000

The data of table 5 are very interesting in that an extremely low solubility is shown. The indications are that soils may be less active because of lack of weathering on the one hand and extreme weathering on the other. The rate of formation of soluble substances in parent glacial material is being investigated and may throw additional light on this question.

Comparative solubility of the separates composing the various soil classes

In a study of the solubility of soils the question naturally arises whether the soluble material is derived from all the particles or largely from the smaller separates.   To throw some light on this phase of the problem a number of soils were divided into their separates and the solubility of each group of particles determined by the same procedure as was used for the soils.   The coarser separates were obtained by passing the soil through a series of screens. Each portion was thoroughly rubbed with a rubber-tipped pestle to insure the crushing of all crumbs.   In addition, after placing the samples in the freezing tubes, all fine material was removed by thoroughly shaking with distilled water and decanting the turbid liquid.   The very fine sand was separated from the silt and clay by sedimentation.   The decanted liquid bearing the silt and clay was allowed to stand about 18 hours and the sediment taken for the portion designated as silt and clay.   No attempt was made to separate the silt and clay since this would involve so much washing that it was feared the results would be vitiated.   The writers realize that these separations were not perfect but are of the opinion that they were sufficiently accurate to warrant the conclusions drawn from the data.

Table 6 contains the data obtained for the 1, 4, 10, 20, 40, and 60-day periods. An examination of the above data reveals the fact that all the separates except the silt and clay have a very low rate of solubility.   Moreover, it seems that the sands from the arid regions are not appreciably more active in this respect than are those from others.   Of course, larger numbers of soils should be run in order definitely to establish this point and additional information will be furnished later.   In this connection the work of Failyer (3), Hall and Russell (4), Loughridge (5), Puchner (8), McCaughey and William (6) and others on the mineralogical and chemical composition of the separates from various soils, is of extreme interest.

This greater solubility of the finer separates may be due to one or more of several causes.   Possibly the greater surface exposed by these minute particles explains the greater rate at which they give up material to solution.   On the other hand, a considerable quantity of salts may be adsorbed by these bodies which are given up at various rates when the particles are subjected to treatment with pure water.   That adsorption enters into the reaction is indicated by the reversion or decrease in the amount of soluble material after a maximum concentration has been reached, which is observed in many cases. It may be true also, that these smaller particles are more completely oxidized, hydrated, or "weathered," and therefore are in reality more highly soluble than the coarser particles.

TABLE 6

Rate of formation of soluble material in the various separates composing a number of soils from different regions of the United States, maintained at 25°C.

	FREEZING-POINT DEPRESSION						
	0 days	1 day	4 days	10 days	20 days	40 days	60 days
	°C.	°C.	°C.	°C.	°C.	°C	°C.
Lubbock, Texas							
Medium sand	0.000	0.000	0.004	0.008	0.009	0.006	0.007
Fine sand	0.000	0.003	0.005	0.010	0.013	0.012	0.009
Very fine sand	0.000	0.006	0.007		0.011	0.012	0.016
Silt and clay	0.000	0.005	0.018		0.030	0.027	0.025
Spur, Texas							
Coarse sand	0.000	0.000	0.002	0.006	0.007	0.008	0.012
Medium sand	0.000	0.000	0.001	0.005	0.008	0.008	0.011
Fine sand	0.000	0.000	0.001	0.005	0.013	0.009	0.011
Very fine sand	0.000	0.000	0.002	0.004	0.015	0.010	0.012
Silt and clay	0.000	0.008	0.012	0.020	0.021	0.021	0.016
Franklin County, Ohio, Ellsworth silt loam							
Coarse sand	0.000	0.000	0.000	0.000	0.003	0.003	0.006
Medium sand	0.000	0.000	0.003	0.000	0.003	0.001	
Fine sand	0.000	0.000	0.002	0.000	0.004	0.002	0.005
Very fine sand	0.000	0.002	0.003	0.005	0.005	0.004	0.005
Silt and clay	0.000	0.005	0.008	0.010	0.010	0.014	0.014
Chillicothe, Texas							
Coarse sand	0.000		0.000	0.000	0.000	0.005	0.004
Medium sand	0.000		0.007	0.001	0.010	0.004	0.002
Fine sand	0.000		0.000	0.006	0.002	0.003	0.003
Very fine sand	0.000		0.003	0.003	0.007	0.007	0.006
Silt and clay	0.000		0.009	0.019	0.019	0.017	0.009
Pecos, Texas							
Medium sand	0.000		0.011	0.010	0.010	0.011	0.012
Fine sand	0.000		0.012	0.002	0.017	0.014	0.016
Very fine sand	0.000		0.004	0.003	0.008	0.008	0.004
Silt and clay	0.000		0.012	0.021	0.020	0.018	0.009
Lind, Washington							
Medium sand	0.000	0.001	0.014	0.011			
Fine sand	0.000	0.001	0.009	0.006	0.007	0.009	0.010
Very fine sand	0.000	0.000	0.003	0.003	0.003	0.003	0.006
Silt and clay	0.000	0.010	0.010	0.014	0.024	0.023	0.023
Pullman, Washington							
Very fine sand	0.000	0.001	0.002	0.007	0.006	0.006	0.006
Silt and clay	0.000	0.011	0.010	0.018	0.028	0.029	0.033
Chochise, Arizona, Sulfur Springs Valley dry farm							
Coarse sand	0.000	0.000	0.004	0.006	0.008	0.006	0.006
Medium sand	0.000	0.002	0.005	0.007	0.008	0.008	0.008
Fine sand	0.000	0.002	0.006	0.007	0.008	0.008	0.008
Very fine sand	0.000	0.000	0.008		0.006	0.005	0.005
Silt and clay	0.000	0.000	0.009		0.017	0.025	0.028

TABLE 6—Continued

	FREEZING-POINT DEPRESSION						
	0 days	1 day	4 days	10 days	20 days	40 days	60 days
	°C.	°C.	°C.	°C. *	°C.	°C.	°C.
Tucson, Arizona, Rillito River bottom							
Coarse sand....................	0.000	0.004	0.002	0.005	0.010	0.006	0.007
Medium sand...................	0.000	0.002	0.002	0.006	0.007	0.010	0.008
Fine sand......................	0.000	0.005	0.005	0.006	0.007	0.013	0.010
Very fine sand.................	0.000	0.000	0.003		0.005	0.007	0.006
Silt and clay...................	0.000	0.017	0.034		0.034	0.034	0.032
Greenville, Kentucky							
Very fine sand.................	0.000	0.002	0.004	0.004	0.004	0.006	0.006
Silt and clay...................	0.000	0.007	0.011	0.010	0.037	0.027	0.021
Lexington, Kentucky							
Coarse sand....................	0.000	0.000	0.001	0.005	0.007	0.005	0.007
Medium sand...................	0.000	0.000	0.002	0.003	0.006	0.003	0.007
Fine sand.....................	0 000	0.000	0.001	0.006	0.006	0.004	0.008
Very fine sand.................	0.000	0.000	0.004	0.004	0.013	0.008	0.008
Silt and clay...................	0.000	0.005	0.009	0.011	0.032	0.021	0.021
Michigan, Miami very fine sand							
Coarse sand....................	0.000	0.000	0.000	0.003	0.002	0.000	0.003
Medium sand...................	0.000	0.000	0.000	0.000	0.003	0.001	0.002
Fine sand......................	0.000	0.000	0.000	0.000	0.004	0.000	0.001
Very fine sand.................	0.000	0.000	0.004	0.003	0.006	0.006	0.004
Silt and clay...................	0.000	0.000	0.015	0.020	0.021	0.019	0.012
Michigan, Miami very light sandy loam							
Coarse sand....................	0.000	0.000	0.001	0.000	0.003	0.002	0.003
Medium sand...................	0.000	0.003	0.000	0.001	0.001	0.000	0.003
Fine sand......................	0.000	0.000	0.002	0.004	0.003	0.000	0.001
Very fine sand.................	0.000	0.004	0.002	0.004	0.007	0.003	0.002
Silt and clay...................	0.000	0.001	0.007	0.018	0.027	0.015	0.011
Michigan, Clyde light silt loam							
Coarse sand....................	0.000	0.000	0.002	0.006	0.008	0.003	0.002
Medium sand...................	0.000	0.000	0.006	0.005	0.005	0.004	0.004
Fine sand......................	0.000	0.000	0.003	0.005	0.005	0.006	0.010
Very fine sand.................	0.000	0.000	0.009	0.010	0.025	0.017	0.014
Silt and clay...................	0.000	0.002	0.027	0.032	0.050	0.044	0.044
Michigan, Miami silt loam							
Coarse sand....................	0.000	0.000	0.000	0.000	0.002	0.003	0.005
Medium sand...................	0.000	0.000	0.000	0.000	0.001	0.003	0.007
Fine sand......................	0.000	0.000	0.000	0.001	0.004	0.004	0.008
Very fine sand.................	0.000	0.003	0.006	0.010	0.008	0.010	0.009
Silt and clay...................	0.000	0.020	0.021	0.028	0.029	0.033	0.014
Michigan, Miami sandy loam							
Coarse sand....................	0.000	0.002	0.002	0.003	0.002	0.003	0.008
Medium sand...................	0.000	0.000	0.002	0.001	0.003	0.004	0.007
Fine sand......................	0.000	0.003	0.001	0.002	0.003	0.005	0.008
Very fine sand.................	0.000	0.003	0.000	0.000	0.000	0.004	0.004
Silt and clay...................	0.000	0.012	0.024	0.022	0.036	0.037	0.038
Nevada Agricultural College							
Coarse sand....................	0.000		0.000	0.001	0.000	0.003	0.003
Medium sand...................	0.000		0.002	0.007	0.006	0.004	0.007
Fine sand......................	0.000		0.011	0.009	0.009	0.013	0.009
Very fine sand.................	0.000		0.012	0.002	0.006	0.008	0.011
Silt and clay...................	0.003		0.020	0.030	0.040		0.043

Effect of grinding on the solubility of the separates

In order to gain some idea of the cause of this greater solubility a number of the coarser separates were ground as finely as could be done conveniently. The grinding was done in a mill with steel burrs which was set so that all the material was small enough to pass a 200-mesh screen.

The separates to be ground were first washed with distilled water until the freezing-point depression was practically zero. As will be observed from the

TABLE 7

Effect of grinding the coarser separates on the rate of formation of soluble material, with the temperature maintained at 25°C.

SAMPLE AND SEPARATE GROUND	FREEZING-POINT DEPRESSION							
	0 days	1 day	3 days	5 days	10 days	20 days	40 days	60 days
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Lubbock, Texas								
Medium sand............	0.008	0.009	0.009	0.009	0.003	0.007	0.012	0.009
Fine sand................	0.009	0.012	0.012	0.015	0.010	0.011	0.011	0.010
Tucson, Arizona, river bottom								
Medium sand............	0.014	0.014	0.016	0.013	0.015	0.016	0.023	0.021
Fine sand................	0.018	0.017	0.020	0.019	0.013	0.015	0.026	0.020
Michigan very light sandy loam								
Coarse sand..............	0.015	0.016	0.019	0.020	0.016	0.021	0.017	0.020
Medium sand............	0.005	0.007	0.008	0.012	0.009	0.012	0.026	0.026
Fine sand................	0.006	0.008	0.009	0.009	0.008	0.010	0.017	0.022
Michigan very fine sand								
Coarse sand..............	0.009	0.010	0.011	0.009	0.015	0.013		
Medium sand............	0.006	0.008	0.009	0.011	0.008	0.009		
Fine sand................	0.006	0.010	0.008	0.008	0.009	0.013	0.016	0.010
Michigan loam								
Coarse sand..............	0.006	0.011	0.014	0.013	0.012	0.011	0.015	0.014
Medium sand............	0.009	0.012	0.015	0.016	0.012	0.012	0.012	0.012
Fine sand................	0.009	0.009	0.016	0.013	0.012	0.016	0.016	0.020
Michigan light silt loam								
Coarse sand..............	0.011	0.013	0.017	0.019	0.018	0.021	0.017	0.024
Medium sand............	0.012	0.011	0.019	0.019	0.018	0.019	0.015	0.028
Fine sand................	0.008	0.010	0.015	0.015	0.013	0.015	0.018	0.018
Michigan, Miami silt loam								
Coarse sand.............	0.009	0.010	0.018	0.013	0.013	0.013		
Medium sand............	0.006	0.008	0.008	0.008	0.008	0.012	0.010	0.010
Fine sand................	0.009	0.010	0.009	0.010	0.010	0.014	0.017	0.018

data (table 7) an appreciable freezing-point depression was observed immediately upon the addition of distilled water, consequently this increased solubility must have been the direct result of grinding. When placed in the constant-temperature chamber further increase in concentration of the soil solution was slight even after 20, 40 or 60 days in most instances. It must be concluded, therefore, that grinding has affected the rate of solubility of the coarser separates, but the influence is not sufficient to warrant the statement that the greater solubility of the silt and clay portion is due entirely to a finer state of division.

Effect of treatment with sodium nitrate on the solubility of soil separates

In the work of Bouyoucos and Laudeman (2) it was found when soils were treated with various salts in solution and then washed until the concentration of the soil solution was practically zero, that the subsequent rate of formation

TABLE 8

Effect of treatment with 0.1 N NaNO₂ on the solubility of the coarser soil separates, maintained at 25°C.

	FREEZING-POINT DEPRESSION						
	0 days	1 day	4 days	10 days	20 days	40 days	60 days
Spur, Texas	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Medium sand....................	0.000	0.009	0.008	0.012	0.011	0.022	0.026
Fine sand......................	0.000	0.008	0.011	0.012	0.014	0.028	0.027
Very fine sand.................	0.000	0.007	0.010	0.011	0.013	0.024	0.029
Lubbock, Texas, 1							
Medium sand....................	0.000	0.008	0.010	0.006	0.009	0.014	0.010
Fine sand......................	0.000	0.010	0.010	0.011	0.012	0.013	0.007
Very fine sand.................	0.000	0.008	0.012	0.012	0.014	0.017	0.003
Ellsworth silt loam, Franklin County, Ohio							
Very fine sand.................	0.000	0.009	0.009	0.011	0.010	0.010	0.008
Lind, Washington							
Very fine sand.................	0.000	0.008	0.007	0.009	0.010	0.007	0.009
Pullman, Washington							
Very fine sand.................	0.000	0.008	0.010	0.011	0.008	0.008	0.010
Cochise, Arizona, dry farm							
Coarse sand....................	0.000	0.003	0.002	0.005	0.006	0.008	0.010
Medium sand....................	0.000	0.008	0.007	0.010	0.008	0.007	0.012
Fine sand......................	0.000	0.008	0.007	0.010	0.008	0.008	0.012
Very fine sand.................	0.000	0.008	0.007	0.010	0.007	0.008	0.012
Tucson, Arizona, river bottom							
Coarse sand....................	0.000	0.006	0.010	0.014	0.018	0.018	0.020
Medium sand....................	0.000	0.007	0.012	0.013	0.015	0.014	0.015
Fine sand......................	0.000	0.007	0.013	0.014	0.015	0.017	0.014
Very fine sand.................	0.000	0.008	0.014	0.017	0.016	0.014	0.012
Greenville, Kentucky							
Very fine sand.................	0.000	0.011	0.010	0.010	0.008	0.016	0.015
Lexington, Kentucky							
Very fine sand.................	0.000	0.010	0.010	0.010	0.009	0.015	0.018
Michigan soils							
Light silt loam							
Coarse sand....................	0.000	0.001	0.009	0.009	0.009	0.008	0.004
Medium sand....................	0.000	0.000	0.008	0.009	0.010	0.008	0.006
Fine sand......................	0.000	0.003	0.008	0.011	0.011	0.009	0.008
Very fine sand.................	0.000	0.004	0.011	0.015	0.015	0.013	0.012
Very light sandy loam							
Coarse sand....................	0.000	0.003	0.003	0.003	0.007	0.001	0.003
Medium sand....................	0.000	0.002	0.003	0.003	0.006	0.001	0.002
Fine sand......................	0.000	0.002	0.003	0.003	0.006	0.002	0.004
Very fine sand.................	0.000	0.002	0.008	0.009	0.010	0.005	0.008

TABLE 8—*Continued*

	FREEZING-POINT DEPRESSION						
	0 days	1 day	4 days	10 days	20 days	40 days	60 days
Very fine sand	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Coarse sand....................	0.000	0.001	0.001	0.003	0.006	0.008	0.004
Medium sand...................	0.000	0.002	0.003	0.003	0.007	0.007	0.005
Fine sand......................	0.000	0.003	0.004	0.003	0.006	0.007	0.006
Very fine sand.................	0.000	0.003	0.008	0.007	0.009	0.009	0.006
Loam							
Coarse sand....................	0.000	0.005	0.008	0.007	0.008	0.009	0.012
Medium sand...................	0.000	0.005	0.007	0.007	0.009	0.007	0.009
Fine sand......................	0.000	0.009	0.007	0.007	0.010	0.008	0.008
Very fine sand.................	0.000	0.008	0.009	0.006	0.012	0.010	0.012
Silt loam							
Coarse sand....................	0.000	0.001	0.000	0.007	0.003	0.005	0.003
Medium sand...................	0.000	0.003	0.004	0.007	0.004	0.007	0.002
Fine sand......................	0.000	0.000	0.003	0.006	0.003	0.006	0.006
Very fine sand.................	0.000	0.006	0.003	0.009	0.006	0.010	0.008

of soluble material was much affected. Some salts markedly increased the rate of solubility while others decreased it. Of the salts tested, sodium nitrate was found to be most active in increasing the rate of solution. It was determined, therefore, to treat the coarser separates of a number of soils with a solution of sodium nitrate. The procedure was as follows. The separates were allowed to stand 24 hours in an 0.1 N solution of sodium nitrate, after which they were washed with distilled water until the freezing-point depression was practically zero. The samples were then placed in tubes in a 25°C. chamber and the freezing point determined at given intervals in the usual manner.

The formation of soluble material is evidently stimulated by treatment with $NaNO_3$. This is not only true for the very fine sand but also for the coarser separates such as a medium sand. The action seems to be quite rapid, as in most cases the major part of the increase occurs within 24 hours. In the case of some soils, however, the effect is more or less continuous to the end of the 60-day period. In general, the separates from the western soils were affected somewhat more than the others studied.

SUMMARY

Comparative rates of formation of soluble substances in surface and subsoils collected from widely separated areas in the United States have been made. Contrary to popular belief it cannot be said from these results that soils formed under conditions of low precipitation are more soluble than are those that have proceeded somewhat farther in their weathering. · Samples of soil taken from formations that have undergone extreme weathering are very inert. It seems, therefore, that so-called new soils are less active than those somewhat older, and the aged soils are almost inert.

The subsoils taken from all regions formed soluble salts very slowly. Our investigations on the soluble-salt content of soils at different depths and periods of the growing season have shown also that there is very little activity in field soils below six inches, that is, in the vicinity of East Lansing, Michigan.

Soil separates comprising a number of soils have been isolated and their activities determined. These results bring out that the finer separates, silt and clay, are responsible chiefly for the formation of soluble salts in the samples of soils under investigations. The sandy particles are very inactive and it cannot be concluded that those taken from soils of arid regions are notably less so than are the others investigated.

Grinding the separates measurably increased their solubility. Usually only slight increases in the material going into solution were observed after the first 24-hour period.

When the separates were treated with 0.1 N $NaNO_3$ and then washed until free of soluble material the rate of formation of soluble substances was measurably affected. Those from the western soils responded somewhat more than the others.

REFERENCES

(1) BOUYOUCOS, G. J. 1919 Rate and extent of solubility of soils under different treatments and conditions. Mich. Agr. Exp. Sta. Tech. Bul. 44.

(2) BOUYOUCOS, G. J., AND LAUDEMANN, W. A. 1917 The freezing-point method as a new means of studying velocity of reactions between soils and chemical agents, and behavior of equilibrium. Mich. Agr. Exp. Sta. Tech. Bul. 37.

(3) FAILYER, G. H., ET AL. 1908 The mineral composition of soil particles. U. S. Dept. Agr. Bur. Soils Bul. 54.

(4) HALL, A. D., AND RUSSELL, E. J. 1911 Soil surveys and soil analyses. In Jour. Agr. Sci., v. 4, p. 2, 182–223.

(5) LOUGHRIDGE, R. H. *1874 On the distribution of soil ingredients among sediments obtained in silt analyses. In Amer. Jour. Sci., v. 7, p. 17.

(6) McCAUGHEY, W. G., AND WILLIAM, H. F. 1913 The microscopic determination of soil-forming minerals. U. S. Dept. Agr. Bur. Soils Bul. 91.

(7) MILLAR, C. E. 1920 A comparative study of cropped and virgin soils. In Mich. Agr. Exp. Sta. Quart. Bul., v. 2, no. 4, p. 195.

(8) PUCHNER, DR. 1907 Ueber die Verteiling von Nährstoffen in den verschieden feinen Bestandteilen des Bodens. In Landw. Vers. Stat., Bd. 66, p. 463–470.

(9) SPURWAY, C. H. 1919 The effect of fertilizer salts treatments on the composition of soil extracts. Mich. Agr. Exp. Sta. Tech. Bul. 45.

THE EFFECT OF CERTAIN ENVIRONMENTAL CONDITIONS ON THE RATE OF DESTRUCTION OF VANILLIN BY A SOIL BACTERIUM

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One of the theories to explain why soils are or become infertile is the soil toxin theory which assumes that the failure of crops to grow on a soil is due to the presence of organic material in the soil which is injurious to the plants. Organic compounds exceedingly injurious to plants have been isolated from the soil. Among these compounds are the aldehydes, vanillin (10) and salicylic aldehyde (9). It has been shown that in at least some Alabama soils, the addition of vanillin has almost no injurious effects on plants (3) because it is rapidly destroyed by soil bacteria (4). It has also been shown that this is true of other soils to which the addition of vanillin has little or no effect on the growth of plants (6). It is true of some soils, however, that although the vanillin-destroying bacteria are present, added vanillin will persist and evidence its injurious effects on the growth of plants for a considerable period of time (6, 11). This persistence must be due to conditions which prevent the bacteria from acting on vanillin. In the present paper a study is made in solution cultures of the effect of certain conditions such as acidity, alkalinity, aeration and mineral salts on the decomposition of vanillin. The experimental work was performed in 1916 and the results, though fragmentary, are presented because conditions have prevented further investigation by the writers.

In order to study this question the vanillin-destroying bacterium isolated from Alabama soil was grown in synthetic culture solutions. What appears to be the same organism has also been isolated from Nebraska, New York and Virginia soils.

No complete study of this organism has been made. It is characterized by its ability to destroy vanillin and by its growth on beef-extract agar. On beef-extract agar, plus 1, it produces a cheesy yellow growth, wrinkled on the surface, and stains the medium yellow. It is aerobic, liquifies gelatine, grows moderately on potato and develops in peptone solutions containing dextrose, lactose, saccharose or glycerine without gas formation. It clears milk without coagulation.

No attempt has been made to determine how many species of bacteria there are which destroy vanillin. Two species have been isolated from the

soil, the one already referred to above and a second which produces no pigment on beef-extract agar. It is believed, however, that the ability to destroy vanillin is not a common property of soil bacteria and that the number of species which have the ability is limited.

<div style="text-align:center">DETERMINATION OF VANILLIN</div>

Early in the study of the action on vanillin of the vanillin-destroying bacterium described above, it was found that the determination of the amount of vanillin by the method used was unreliable. The method first used was that described by Folin and Denis (2) with clarification by the lead acetate omitted. This method requires a reagent composed of phosphoric acid, sodium tungstate and phospho-molybdic acid and is referred to by them as the phenol reagent. On the addition of a saturated sodium carbonate solution, it produces with vanillin a deep blue solution, the depth of the color varying directly with the amount of vanillin present. Using the phenol reagent to determine the amount of vanillin in a synthetic nutrient solution in which the vanillin-destroying bacterium had grown, it was sometimes found that apparently more vanillin was present after the action of the bacterium than had been originally added. In some cases, twice as much vanillin was apparently formed in the inoculated culture as was present in an uninoculated, or check culture.

In an attempt to clear up this contradiction, the acid nitrate of mercury described by Estes (1) was used for determining vanillin. This reagent produces with vanillin a pink color, the depth of color varying directly with the amount of vanillin present. It was found that, determined by this means, the amount of vanillin progressively decreased in a synthetic nutrient solution containing vanillin and inoculated with a pure culture of the vanillin-destroying bacteria.

On further investigation, it was found that the anomalous results secured by the use of the phenol reagent were due to the fact that the bacterium oxidizes vanillin to vanillic acid (5) which produces a blue color with the phenol reagent 1.5–2.0 times as strong as is produced by an equal amount of vanillin. The depth of color yielded by a mixture of vanillin and vanillic acid with the phenol reagent, therefore, is the result of the color produced by both the vanillin and vanillic acid. The color production of vanillic acid with the Estes reagent however, is very slight so that vanillin can be determined in a mixture of vanillin and vanillic acid by the use of that reagent. Acid nitrate of mercury was therefore used for the determination of vanillin in the experiments reported below.[1]

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[1] It is probable that, by the use of lead acetate, a solution containing both vanillin and vanillic acid could be freed of the vanillic acid as it is precipitated by lead acetate in the presence of ammonia. The phenol reagent could then be used for the determination of the vanillin.

EFFECT OF ACIDITY AND ALKALINITY ON THE DECOMPOSITION OF VANILLIN

In determining the effect of acidity and alkalinity on the destruction of vanillin, the following nutrient solution was used:

K_2SO_4	0.0340 gm.
$NaNO_3$	0.1000 gm.
$CaH_4(PO_4)_2$	0.0710 gm.
Distilled Water	3000 cc.
Vanillin	As indicated

The solution was neutralized to phenolphthalein with NaOH, and aliquot parts made acid and alkaline with HCl and NaOH as noted in table 1. Forty cubic centimeters of solution were placed in 150-cc. Erlenmeyer flasks and after sterilization, 10 cc. of a sterile vanillin solution were added to each flask.[2] The organisms were allowed to grow 4 days at room temperature when the vanillin was determined by means of the acid nitrate of mercury reagent. Previous to the determination of the vanillin, the contents of each flask were neutralized.

TABLE 1

Effect of HCl and NaOH on the decomposition of vanillin

ADDITIONS PER 100 CC. OF SOLUTION	VANILLIN REMAINING IN CULTURE SOLUTION AT END OF 4 DAYS
	p.p.m.
10 cc. 0.1 *N* NaOH	260.0
5 cc. 0.1 *N* NaOH	41.6
2 cc. 0.1 *N* NaOH	24.0
1 cc. 0.1 *N* NaOH	23.6
Neutral to phenolphthalein	28.0
1 cc. 0.1 *N* HCl	274.0
2 cc. 0.1 *N* HCl	226.0
Checks, uninoculated	241.6

In this experiment which is the average of duplicate cultures, 1 cc. of 0.1 *N* HCl per 100 cc. was sufficient to inhibit the growth and action of the bacterium on vanillin. Between 5 and 10 cc. of 0.1 *N* NaOH were required, however, to stop its action. In this connection, it should be noted that Skinner and Noll (12) report that on unproductive soil the toxic effects of vanillin are overcome by liming. Truog and Sykora (13) also found that in an infertile acid sand, the poisonous action of the vanillin was greatly lessened by the addition of lime. A study of the relation of hydrogen-ion concentration to the decomposition of vanillin in solution cultures and sand should be made.

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[2] This method of adding vanillin in solution after sterilization was used throughout when quantitative work was done, as it eliminated any danger of lack of uniformity in vanillin content of the flasks due to sterilization.

EFFECT OF AERATION ON THE RATE* OF DECOMPOSITION OF VANILLIN

While studying the formation of the decomposition products of vanillin, it was observed that in a 2-liter Erlenmeyer flask containing 250 cc. of nutrient solution, the vanillin as tested for by the acid nitrate of mercury would disappear in three or four days. In the same flask, however, containing 1000 cc. of solution, vanillin was present after 3 weeks. The depth of solution in this case was about 7 cm. This difference was thought to be due to aeration. It should be noted, however, that the flask in which the slower digestión occurred had four times as much vanillin in it though the concentrations were the same.

To determine more definitely the effect of the depth of the solution on the rate of the disappearance of vanillin under the action of the vanillin-destroying bacterium, experiments were conducted in which 50 cc. of nutrient solution, made −0.2 Fuller's scale with NaOH, were placed in 2.5 by 25-cm. test-tubes and 150-cc. Erlenmeyer flasks. The quantity and composition of

TABLE 2

Effect of aeration on the rate of digestion of vanillin

Depth of solution in flask about 2 cm., in tube about 12 to 14 cm.

TIME FROM INOCULATION	VANILLIN IN FLASK		VANILLIN IN TUBE	
	Check not inoculated	Inoculated	Check not inoculated	Inoculated
days	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
3	242	108	226	190
5	210	24	220	78
7	306	8	292	40
9	234	0	224	34
11			246	14

the solution in both tubes and flasks were identical but in the tubes the depth of solution was 12 to 14 cm. with a diameter at the surface of the liquid of 2.5 cm. while in the flask the depth was about 2.0 cm. and the diameter at the surface of the liquid was 6.5 cm.

It is evident from the data in table 2 that the depth of the solution and the area of liquid surface exposed affect the rate of the decomposition of vanillin by this bacterium. The more rapid decomposition occurs in the flask where the depth of the solution is one-sixth of that in the tube and the liquid surface exposed is $2\frac{1}{2}$ times as great. This difference is no doubt due to the freer supply of oxygen offered in the case of the flask and is what would be expected when the destruction of vanillin by the organism is known to be an oxidative process.

THE EFFECT OF FERTILIZER SALTS ON THE DECOMPOSITION OF VANILLIN

It has been shown by Schreiner and Skinner (8) that fertilizer salts affect the toxicity of vanillin to wheat. The effects of calcium acid phosphate, sodium nitrate and potassium sulfate singly and in many combinations on

the toxicity of vanillin were studied by these investigators. The growth of wheat plants in culture solutions respectively high in phosphate, nitrate or potash and containing vanillin, showed that the vanillin depressed the growth least in the cultures high in nitrate and most in the cultures high in potash.

The writers have attempted to discover whether the same fertilizer salts have any effect on the rate at which vanillin is decomposed by the bacterium used in this investigation. The triangular diagram as used in physical chemistry was employed and the results are presented by its means. Monobasic calcium acid phosphate, sodium nitrate and potassium sulfate were used in 15 different combinations and in such concentrations that each culture solution contained a total concentration of 80 parts per million of P_2O_5, NH_3 and K_2O. The mineral combinations used in parts per million of K_2O, P_2O_5 and NH_3 were as follows:

	K_2O	P_2O_5	NH_3
1	0	80	0
2	0	60	20
3	0	40	40
4	0	20	60
5	0	0	80
6	20	60	0
7	20	40	20
8	20	20	40
9	20	0	60
10	40	40	0
11	40	20	20
12	40	0	40
13	60	20	0
14	60	0	20
15	80	0	0

In preparing the culture solution sufficient of each of the three chemically pure salts was dissolved in distilled water to produce solutions containing 100 parts per million of P_2O_5 and K_2O, respectively.

This required:

> 0.1776 gm. of $CaH_4(PO_4)_2.H_2O$ per liter
> 0.5000 gm. of $NaNO_3$ per liter
> 0.1852 gm. of K_2SO_4 per liter

To prepare solution no. 1, 40 cc. of the $CaH_4(PO_4)_2$ were placed in a 150-cc. Erlenmeyer flask and after sterilization 10 cc. of a sterile vanillin solution were added, making a total of 50 cc. To prepare solution no. 7, 10 cc. of the K_2SO_4, 20 cc. of the $CaH_4(PO_4)_2$ and 10 cc. of the $NaNO_3$ stock solution were placed in a 150-cc. Erlenmeyer flask and after sterilization, 10 cc. of a sterile vanillin solution were added.

Three separate experiments were performed. In each experiment the combinations of $CaH_4(PO_4)_2$, K_2SO_4 and $NaNO_3$ noted above were used. The

concentration of vanillin was uniform throughout a series. In experiment 1 (see table 3), 150-cc. Erlenmeyer flasks containing 50 cc. of solution were used and the concentration of vanillin was 508 parts per million. In experiment 2, the same containers and amount of solution were used but the experiment was performed in duplicate and the concentration of vanillin was 296 parts per million. In experiment 3, 50 cc. of solution were used in test-tubes approximately 2.5 by 25 cm. and the concentration of vanillin was 260 parts per million. Growth occurred at room temperature, the time varying with the experiment from 3 to 5 days. At the close of the experiment, the residual vanillin was determined by means of the acid nitrate of mercury reagent.

TABLE 3

Effect of fertilizer salts on the rate of decomposition of vanillin

SOLUTION	VANILLIN REMAINING IN CULTURES			
	Experiment I	Experiment II	Experiment III	Average
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	48.0	148.0	37.0	77.6
2	18.8	108.0	60.0	62.3
3	16.8	102.0	100.0	72.9
4	32.0	114.0	96.0	80.6
5	19.2	180.0	128.0	109.1
6	56.4	110.0		83.2
7	40.4	116.0	62.0	72.8
8	167.6	118.0	118.0	134.5
9	164.0	152.0	80.0	132.0
10	532.0*	106.0	188.0	147.0
11	Trace	130.0	254.0*	65.0
12	176.0	160.0	228.0	188.0
13	80.0	146.0	36.0	87.3
14	54.0	242.0	12.0	102.7
15	160.8	146.0	156.0	154.3
Checks	508.0	296.0	260.0	354.7

* No decomposition. Omitted from averages.

The results indicate that the decomposition of vanillin proceeds most rapidly in those combinations of salts high in $CaH_4(PO_4)_2$. The complete data are given in table 3 where the amount of vanillin in parts per million remaining in each culture solution at the end of the experiment is given.

Using the averages for the three experiments given in column 4 of table 3, the total of the amounts of vanillin remaining in all those combinations of salts containing 50 per cent of $CaH_4(PO_4)_2$ or more, is 515.8 parts per million; in all those combinations of salts containing 50 per cent of $NaNO_3$ or more, is 717.1 parts per million; and in all those combinations containing 50 per cent of K_2SO_4 or more, is 744.3 parts per million.

It is evident that the decomposition of vanillin in these experiments proceeds most rapidly in the cultures high in $CaH_4(PO_4)_2$ and least rapidly in

the cultures high in K_2SO_4. This fact is shown graphically in figure 1. The 15 cultures are here arranged on the triangular diagram. Each circle on the diagram represents a culture, the upper numbers indicating the solution and the lower numbers the amount of vanillin remaining in the solution after the action of the bacteria. The solutions at the corners of the diagram contain one salt only; cultures 1, 5 and 15 containing $Ca_2H_4(PO_4)_2$; $NaNO_3$ and K_2SO_4, respectively. The cultures on the sides of the diagram contain combinations of two of
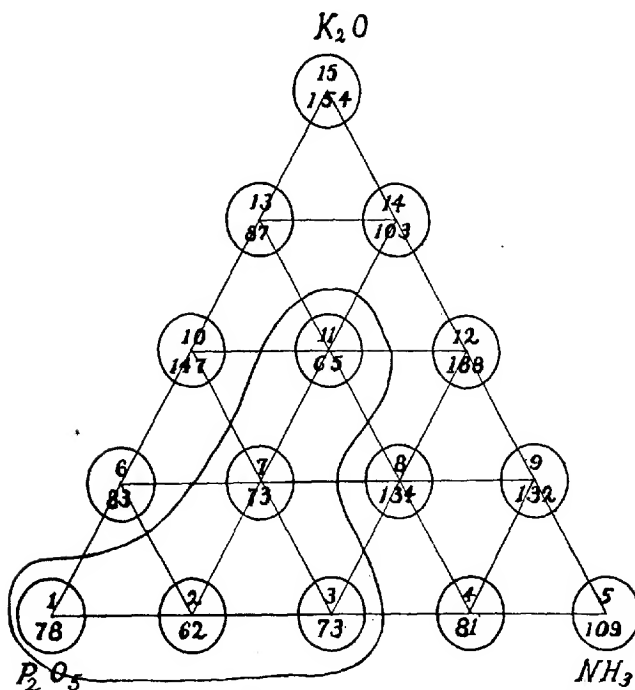
FIG. 1. TRIANGULAR DIAGRAM SHOWING THE EFFECT OF FERTILIZER SALTS ON THE RATE OF DECOMPOSITION OF VANILLIN

the salts and the cultures within the diagram contain combinations of the three salts in the proportions indicated earlier. An examination of the amounts of residual vanillin in the cultures shows that the smallest amounts are located in the corner of the triangle where the concentration of phosphate is highest. This is indicated in the figure by surrounding all those cultures containing less than 80 parts per million of residual vanillin by a line.

Although the effect of $CaH_4(PO_4)_2$ on the rate of decomposition of vanillin is marked, it is impossible at the present stage of the investigation to state

the cause of its effect.   Several explanations are possible.   It may be that the balancing effect of the calcium on the other salts in the solution permits a greater growth of the bacteria and therefore greater digestion of vanillin in those solutions high in calcium.   It may be a specific effect of the phosphate radical.   The decomposition of vanillin by this organism is an oxidative process.   Schreiner and Sullivan (7) have shown that phosphates and nitrates increase the oxidative power of roots.   In this connection it is interesting to note that the same investigators in studying the effect of the addition of sodium nitrate, potassium sulfate and calcium acid phosphate to the soil on the oxidative power of the soil, found that of the three salts acid calcium phosphate produced the greatest increase in oxidation in four soils out of five (table 15) and was greatest or second greatest in effect in all but one trial with a second lot of four soils (table 14).

EFFECT OF GLUCOSE ON THE DECOMPOSITION OF VANILLIN

To determine whether the presence of an easily fermentable carbohydrate such as glucose would affect the action of the bacterium on vanillin, experiments were conducted in synthetic nutrient cultures to which both vanillin and glucose had been added.   It was found that the vanillin and glucose were used by the bacterium simultaneously and that the effect of the glucose on the destruction of vanillin was not marked.

DISCUSSION

In the work of which the present paper is the fourth report, we have tried to determine why organic toxic compounds were injurious to plants when added to some soils but not injurious when added to others.   Previous investigators have assigned the more important role in the amelioration of the toxic effect of organic compounds to the physical action of the soil, to the chemical reactions which take place in the soil between the toxins and soil constituents or to the oxidative and reducing ability of plant roots.   We have found that by far the most important role in the amelioration of the toxicity of organic compounds in the soil is played by the destructive action of soil microorganisms; in some cases by the action of specific bacteria.   It is a well recognized fact that the ability to destroy some organic compounds is not a common property of all bacteria but for many organic compounds there are a limited number of species which have the power of destroying them.   The complete distintegration of a mass of dead plants or animals is not accomplished by one species of microorganisms but is accomplished by the successive action of many.   One group acts on the end-products of another.   If at any stage the suitable organisms are not present or conditions are such that they cannot act, then the material which they usually break down will accumulate. This accumulation may be only temporary, disappearing with a change in conditions, or it may be more permanent, as in humus formation and the

formation of peat. But in any case, the destruction or accumulation is basically a function of the action of microorganisms.

While it is impossible to apply too strictly to the soil the results from solution cultures, we have shown in the present paper that alkalinity and good aeration favor the destruction of vanillin, because of their effect on the action of the vanillin-destroying bacteria on vanillin. We have also shown that some of the salts commonly used as fertilizer elements affect the rate at which vanillin is destroyed by bacteria. If it can be shown that the opposite conditions, acidity, poor aeration and the abundance or deficiency of some mineral salts allow the formation of vanillin to proceed, then the conditions for its accumulation and action as a factor in soil fertility are defined.

SUMMARY

1. It is believed that the number of species of soil bacteria able to destroy vanillin is limited.

2. Slight concentrations of HCl inhibit the action on vanillin of the soil bacterium studied.

3. Aeration favors the destruction of vanillin by the organism used.

4. In solution cultures containing $CaH_4(PO_4)_2$, $NaNO_3$ and K_2SO_4 singly or in combination and inoculated with the bacterium used, vanillin is destroyed most rapidly in those solutions high in $CaH_4(PO_4)_2$ and least rapidly in the solutions high in K_2SO_4.

5. The presence of glucose has no marked effect on the rate at which vanillin is destroyed by the bacterium used.

REFERENCES

(1) ESTES, C. 1917 A new qualitative test and colorimetric method for the estimation of vanillin. *In* Jour. Indus. Engin. Chem., v. 9, no. 2, p. 142–144.

(2) FOLIN, O., AND DENIS, W. 1912 A new colorimetric method for the determination of vanillin in flavoring extracts. *In* Jour. Indus. Engin. Chem., v. 4, no. 9, p. 670–672.

(3) FUNCHESS, M. J. 1916 The effects of certain organic compounds on plant growth. Ala. Agr. Exp. Sta. Bul. 191, p. 103–132.

(4) ROBBINS, W. J. 1917 The cause of the disappearance of cumarin, vanillin, pyridine and quinoline in the soil. Ala. Agr. Exp. Sta. Bul. 195, p. 49–64.

(5) ROBBINS, W. J., AND LATHROP, E. C. 1919 The oxidation of vanillin to vanillic acid by certain soil bacteria. *In* Soil Sci., v. 7, p. 475–485.

(6) ROBBINS, W. J., assisted by A. E. ELIZANDO. 1918 The destruction of vanillin in the soil by the action of soil bacteria. Ala. Agr. Exp. Sta. Bul. 204, p. 125–131.

(7) SCHREINER, O., AND SULLIVAN, M. X. 1910 Studies in soil oxidation. U. S. Dept. Agr. Bur. Soils Bul. 73, p. 1–57.

(8) SCHREINER, O., AND SKINNER, J. J. 1911 Organic compounds and fertilizer action. U. S. Dept. Agr. Bur. Soils Bul. 77.

(9) SHOREY, E. C. 1913 Some organic soil constituents. U. S. Dept. Agr. Bur. Soils Bul. 88.

(10) Shorey, E. C. 1914 The presence of some benzene derivatives in soils. *In* Jour. Agr. Res., v. 1, no. 5, p. 357–363.

(11) Skinner, J. J. 1915 Field tests with a toxic soil constituent, vanillin. U. S. Dept. Agr. Bul. 164.

(12) Skinner, J. J., and Noll, C. F. 1916 Field tests of fertilizer action on soil aldehydes. *In* Jour. Amer. Soc. Agron., v. 8, p. 273–298.

(13) Truog, E., and Sykora, J. 1917 Soil constituents which inhibit the action of plant toxins. *In* Soil Sci., v. 3, no. 4, p. 333–351.

NOTES ON THE CONFERENCE ON ELEMENTARY SOIL TEACHING, HELD AT LEXINGTON, KENTUCKY, JUNE, 1920

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Representatives of the agronomy or soils departments of sixteen state agricultural colleges met at the University of Kentucky on June 23, 24, and 25 to discuss the teaching of the first, or general, soil courses. The entire field of the teaching of these courses was considered but the attention of the conference was directed in particular to the securing of greater uniformity in the giving of the work in the various colleges and to the determination of what should properly constitute the laboratory part of this work.

The conference unanimously recommended that the first, or elementary, soils work should be given in a uniform general course dealing largely with the scientific principles underlying the successful management of soils with such practical application as good teaching and local conditions demand; this course to be required of all agricultural students, to be taken in the sophomore year when practicable, and to carry approximately 5 semester hours credit.

The minimum prerequisites recommended for this course were one year of general inorganic chemistry, one term of general geology and either high-school or college physics.

Further recommendations were that the subject matter of the course should be presented in well correlated lecture, recitation and laboratory work, that at least three-fifths of the time should be spent in lecture and recitation, and that it is desirable that a standard text book be used.

A suggested general outline of laboratory exercises was worked out. In this outline, for the most part, the common stock exercises are omitted. Exercises involving mainly quantitative work also are largely omitted if the laboratory work is confined to one credit hour per week.

The advantages of such a course, as brought out in the discussion, are that the student is enabled to obtain a survey of the entire subject in one course, that the preparation of standard text books, illustrative material, and standard laboratory equipment will be made possible and that transfer of credits from one institution to another will be facilitated.

The institutions represented at the conference were: University of Maryland, Georgia State College of Agriculture, Louisiana State University, University of Kentucky, Pennsylvania State College, University of Tennessee, Cornell University, Montana State College, University of Missouri, University of Illinois, A. & M. College of Texas, Michigan Agricultural College, University of Vermont, Ohio State University, Virginia Polytechnic Institute, University of Nevada.

NITROGEN ECONOMY IN THE SOIL AS INFLUENCED BY VARIOUS CROPS GROWN UNDER CONTROL CONDITIONS

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During recent years and especially under present conditions, the high price of fertilizer constituents has necessitated in some cases other means of maintaining the fertility of the soil than by applying the customary amounts of commercial fertilizer. The constituent that this paper has to deal with, viz., nitrogen, has become quite expensive; consequently more and more attention is devoted to efforts to maintain the nitrogen supply in the soil through various cultural practices.

One practice, which is a very ancient one, is the growing of various leguminous crops as green manure to be plowed under. That leguminous crops when plowed under always add nitrogen to the soil in excess of that removed by the crop in its growth has apparently seldom been questioned. Likewise, it apparently has generally been taken for granted that a non-leguminous green manure, when turned under, always returns to the soil all the nitrogen that has been removed during its growth.

It is obviously difficult to measure by chemical analysis any slight change in the nitrogen content of a field soil following any treatment due to many uncontrollable factors. The customary method of measuring the effect of a treatment is by comparison in the following crop. In this case evidence does not always show that a leguminous green-manure crop turned under gives a measurable advantage over a non-leguminous green manure, as A. J. Pieters has pointed out in the general summary of his review of American Experiment Station Literature on Green Manures (4). It is further claimed by some that the legume, even when harvested and removed from the soil, leaves as much nitrogen in the roots left behind as was present in the original soil. In other words, it is their claim that the legume in its growth takes from the air, through symbiotic fixation by the legume bacteria, as much nitrogen as is found in that part of the plant above the ground; and that, therefore, when the plant has been harvested the soil has not been robbed but the nitrogen equilibrium has been maintained. On the contrary C. O. Swanson (7) reports that in a number of alfalfa fields under investigation in Kansas which have been cropped continuously for 20 to 30 years, in no field is the nitrogen content equal to that in near-by native sod, except in a few instances in the semi-

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arid portion of the state where it is greater.   That there are constantly occur-
ring losses of nitrogen in cultivated soil over and above that removed by crops
has been brought out by Russell in his monograph "Soil Conditions and Plant
Growth" (5).   At Rothamsted a small plot of land was isolated in 1870 and
thereafter kept free from vegetation by hoeing, disturbing the soil as little
as possible.   This plot was afterward converted into a lysimeter by surround-
ing it with a cement wall and underdraining.   The drainage water was ana-
lyzed daily.   During the last twenty years of the experiment, all analyses
were made by the same analyst.   He found that the soil lost from 1870 to
1905 nitrogen equivalent to 1050 pounds per acre.   He further points out
that all but 110 pounds of this was recovered as nitrate in the drainage water.
The remaining 110 pounds was probably dissipated as free nitrogen or ammo-
nia.   Shutt (6) reports a loss of nitrogen in a Saskatchewan Prairie soil after
22 years of cultivation amounting to 2190 pounds per acre.   Of this, 700
pounds were recovered in the crop, thus leaving a dead loss of 1490 pounds.
Since there is practically no drainage water, probably little of this loss was
due to leaching.   Lipman and Blair (2) and Mooers (3) in their cylinder ex-
periments both report a gradual decrease in nitrogen both under crops and
under fallow.   Some of this may be due to leaching, but viewed in the light
of the experiment at Rothamsted already mentioned and the work reported
upon in this paper, it is safe to conclude that in cropped, cultivated, or other-
wise disturbed soil there is certain loss of nitrogen through volatilization.
Cultivation is probably responsible for the loss of considerable of the soils'
store of nitrogen.   The fact can hardly be disputed that the excessive aeration
incidental to cultivation causes the oxidation of much organic matter and the
consequent liberation of much nitrogen probably in its elemental form.   This
condition is quite evident in old orchards that have been subjected for a num-
ber of years to clean cultivation.   The soil gradually becomes "lifeless"
through loss of organic matter which must be supplied along with some form
of nitrogen in increasing amounts to maintain the original vigor of the trees.
Results published by the author (8) show a steady loss in total nitrogen in a
number of instances where soils were kept in the laboratory at optimum mois-
ture content and thoroughly stirred and sampled periodically.

PLAN OF WORK

A series of pot experiments were started by the author at Arlington Farm of
the United States Department of Agriculture in 1914.   The object in view
was to make a comparative study of the amount of nitrogen removed from the
soil by representative leguminous and non-leguminous crops grown under
control conditions, and of the amount of nitrogen recovered in these crops
themselves.   In brief, the procedure was as follows.   The crops were grown
in heavy galvanized buckets 15 inches in diameter by 13 inches deep.   These
buckets hold from 100 to 120 pounds, depending on the character of the soil.

All were housed in a cage built of 1-inch iron pipe covered with wire netting. The buckets were watered to weight daily with distilled water. Detailed description of the construction of the cage and the handling of the containers is given in a paper by the author (9) in the Journal of the American Society of Agronomy.

The soil used was first stirred thoroughly by being screened and then shoveled over several times on a cement mixing-floor. Equal quantities were then weighed out into the containers. A sample of this soil was immediately dried out and retained as a dry check. Fallow checks were kept and treated the same throughout as planted soils. On reaching maturity each crop was harvested close to the surface of the soil, dried, weighed, and ground fine for analysis of total nitrogen. The roots were then screened out of the soil and after being dried and ground were returned and thoroughly mixed with the soil in each container to allow a uniformly representative sample to be taken for nitrogen determination. Samples of soil were then removed and air-dried for analysis. The fallow checks were handled and sampled the same as the cropped soils.

All nitrogen results reported constitute the average of two closely agreeing determinations on 1-gram samples of crops and 10-gram samples of soil. Determinations on crop samples were made by the Gunning method and on soil by the Kjeldahl-Gunning-Jodlbauer method. With both methods the sulfate mixture was used as recommended by Lipman and Sharp (1, p. 648). Nitrates were determined by a modification of the Ulch method. This operation may be briefly described as follows. One hundred grams of air-dry soil is shaken at frequent intervals for half an hour with 285 cc. of distilled water and 15 cc. of aluminum cream. The extract is then filtered off, measured and acidulated with 3 cc. of sulfuric acid. About 2 gm. of iron dust is then added and reduction allowed to take place over-night in the cold. Approximately 8 gm. of heavy magnesium oxide is then added and ammonia distilled off in the usual way. Since only a trace of ammonia was ever found in the soil this was not expelled from the solutions before reduction was started.

In 1914 the soil used was a clay which had been composted with manure and left in a pile for several years previous. A quantity of this soil was limed and portions representing 45 kgm. when brought to the optimum moisture condition were weighed into the containers. The crops grown this season were spring oats, barley, rye, kafir corn, field corn, pearl millet, sugar beets, hairy vetch, field peas, and red clover. All were grown in quadruplicate. The number of plants grown per bucket were four of kafir corn, sugar beets, and millet; three of corn, and ten of all the rest.

In table 1 is shown the yield expressed in grams of dry matter of the entire crop from each bucket.

Table 2 and figure 1 show the yield in grams of nitrogen from each crop and the grams of nitrogen remaining in the soils after the removal of each crop; also the nitrogen found in the fallow check soils and in the dry check which represents the nitrogen in the soil at the beginning of the experiment.

TABLE 1

Dry weights of crops harvested

CROP	WEIGHT	AVERAGE WEIGHT
	gm.	*gm.*
Millet................................	320.0 308.0 309.0 297.1	308.5
Corn.................................	335.5 309.3 299.2 339.5	320.9
Kafir corn...........................	338.9 330.8 401.5 357.0	357.0
Oats.................................	147.7 146.7 145.9 142.2	145.6
Wheat................................	74.2 74.0 69.5 70.7	72.1
Barley...............................	106.5 127.5 127.3 111.4	118.2
Rye..................................	48.2 47.7 41.0 37.0	43.5
Sugar beets..........................	224.5 232.7 247.0 225.8	232.5
Red clover	83.5 96.0 101.3 95.3	94.0

TABLE 1—*Continued*

CROP	WEIGHT	AVERAGE WEIGHT
	gm.	*gm.*
Vetch	81.2 98.0 69.0 61.9	77.5
Soybeans	329.3 300.8 313.7 337.0	320.2
Field peas	77.7 50.1 10.0 59.3	49.3

From a study of the table and figure the following facts are brought out. Millet and corn removed practically equal amounts of nitrogen from the soil and equal amounts were recovered in the crops; practically as much nitrogen being recovered as was originally present in the soil. Kafir corn, which produced somewhat more dry matter than either millet or corn, also removed more nitrogen from the soil, however in this case not so much was recovered in the crop. Slightly more nitrogen was recovered from oats than from kafir corn. Practically the same amount of nitrogen was recovered from wheat, barley, rye, beets and clover; this amount being less than with the first-named crops, and about 1 gm. less than was originally present in the soil. Somewhat more was recovered from vetch and field peas than in the preceding group. With soybeans more nitrogen was recovered than was originally present.

1. In none of the soils excepting that growing soybeans was as much nitrogen recovered as was present in the original soil represented by the dry check.

2. More nitrogen was lost by some crops than by others.

3. More nitrogen was lost from the fallow soils than from any of the cropped soils. In the latter most of the nitrogen removed by the crops was recovered.

4. Among the legumes only soybean plants contained more nitrogen than was drawn from the soil, considerable nitrogen in this case being fixed from the atmosphere.

In table 3 is shown the nitrogen as nitrate found in the soils after harvest. These results are presented in parts per million and in grams per bucket. On comparing these results with those in table 1 it is evident that in every case the nitrate found after harvest is indirectly proportional to the yield of dry matter. The largest amount of nitrate was naturally found in the fallow check.

TABLE 2

Total nitrogen remaining in the soil and recovered in crops after harvest

CROP GROWN	SOIL		CROPS		TOTAL NITROGEN RECOVERED
	Nitrogen per bucket	Average nitrogen	Nitrogen per crop	Average nitrogen	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Dry check.........	55.14	55.14			55.14
Fallow check......	52.84	53.25			53.25
	52.84				
Pearl millet.......	52.39	52.89	2.12	2.10	54.99
	53.08		2.20		
	53.12		2.06		
	52.98		2.04		
Corn..............	53.12	52.93	2.21	2.15	55.08
	52.50		1.98		
	52.15		1.99		
	53.94		2.43		
Kafir corn.........	52.15	51.54	2.66	2.88	54.42
	51.29		2.76		
	51.19		3.41		
	51.53		2.71		
Oats..............	50.81	52.12	2.50	2.44	54.56
	51.81		2.49		
	53.46		2.40		
	52.70		2.37		
Wheat............	53.46	52.47	1.39	1.36	53.83
	52.15		1.40		
	52.26		1.30		
	52.02		1.35		
Barley............	51.54	51.84	2.11	2.16	54.00
	53.46		2.26		
	51.19		2.28		
	51.19		2.01		
Rye..............	54.32	53.26	0.90	0.83	54.19
	53.70		0.91		
	52.46		0.68		
	52.98				
Sugar beets........	50.57	50.30	3.54	3.53	53.80
	50.33		3.58		
	51.06		3.52		
	49.23		3.47		

TABLE 2—*Continued*

CROP GROWN	SOIL		CROPS		TOTAL NITROGEN RECOVERED
	Nitrogen per bucket	Average nitrogen	Nitrogen per crop	Average nitrogen	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Red clover........	51.06	51..35	2.26	2.45	53.80
	52.74		2.49		
	50.81		2.55		
	50.81		2.50		
Hairy vetch.......	52.50	52.16	2.26	2.18	54.34
	53.32		2.71		
	52.39		1.98		
	50.43		1.77		
Soybeans.........	53.26	52.51	8.93	8.42	60.93
	52.64		7.91		
	52.02		8.10		
	52.02		8.73		
Field peas........	52.15	52.80	2.03	1.32	54.12
	53.22		1.30		
	54.42		0.30		
	51.43		1.66		

Figure 2 presents a combination of results, viz., the nitrate nitrogen found in the soil after harvest and that actually used by the crops, assuming that the crops obtained their nitrogen as nitrate. Some quite interesting results are brought out here. With but possibly one exception there was a distinct loss of nitrate nitrogen incidental to cropping compared with that found in the fallow check soil. The crops seemed to be divided loosely into groups thus, wheat, oats, barley and rye, which produced comparatively light yields of dry matter, left comparatively large amounts of nitrate in the soil (in this respect wheat and rye left considerably more than oats or barley) and this added to that recovered in the crops make a total of recovered nitrate considerably greater than that from millet, corn and kafir corn. In this latter ground the yield of dry matter was roughly four times greater than in the former, and the nitrate recovered was the least of any of the other crops. Sugar beets might be classed with the preceding group with respect to the nitrate left in the soil, but in the total nitrate recovered they compare closely with the first group. Clover and vetch, which in yield of dry matter exceeded somewhat wheat and did not equal that of oats and barley, left considerably less nitrate in the soil than either of these and the total nitrate recovered was less than for any other except rye. Field peas compared closely with wheat. With soybeans there was a fixation of total nitrogen, so it is uncertain how much was obtained from the atmosphere. However, from table 2 and figure 1

it is seen that there was 2.63 gm. of nitrogen taken from the soil when compared with the dry check. This leaves 5.79 gm. of nitrogen which was obtained from the atmosphere. In figure 2, if this 5.79 gm. were subtracted from the portion of the figure represented in the nitrogen contained in soybeans, we
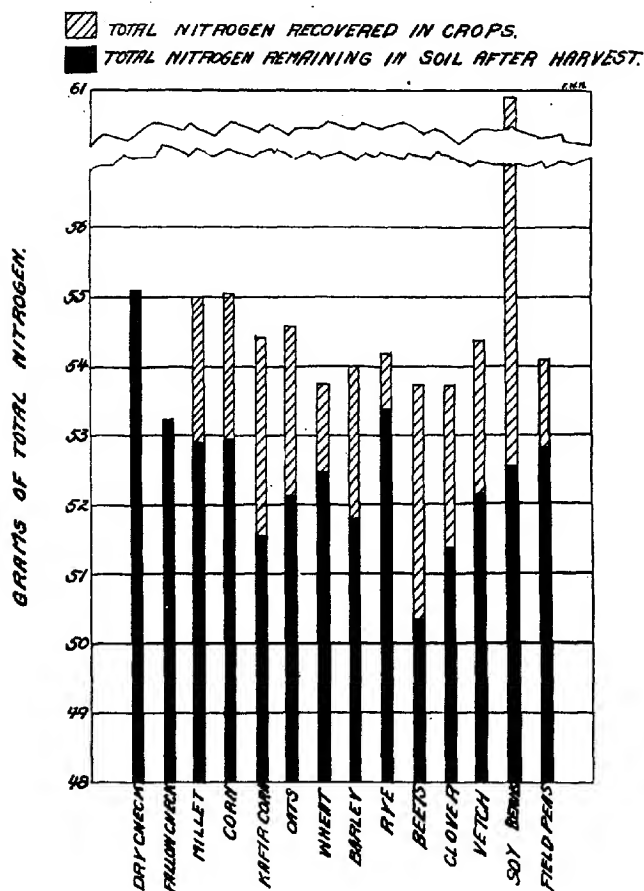
FIG. 1. TOTAL NITROGEN REMAINING IN THE SOIL AND THAT RECOVERED IN THE CROPS AFTER HARVEST

would have left what might represent that portion of the nitrogen in the crop removed from the soil as nitrate together with the nitrate left in the soil, both of which would amount to 3.97 gm., which would also, as in the cases of the other crops, be less than the nitrate formed in the fallow check. This

TABLE 3

Nitrate nitrogen in the soil after harvesting the crops

CROP GROWN	PARTS PER MILLION	AVERAGE	GRAMS	AVERAGE
Fallow check................	148.00	146.0	5.09	5.02
	144.00		4.95	
Millet.......................	27.00	24.5	0.93	0.84
	22.00		0.76	
	26.00		0.89	
	23.00		0.79	
Corn........................	26.00	26.5	0.89	0.91
	31.00		1.07	
	27.00		0.93	
	22.00		0.76	
Kafir corn...................	16.00	19.00	0.56	0.65
	22.00		0.76	
	19.00		0.65	
	19.00		0.65	
Oats........................	42.00	60.00	1.44	2.07
	68.00		2.34	
	59.00		2.03	
	72.00		2.48	
Wheat.......................	95.00	97.00	3.26	3.33
	94.00		3.23	
	102.00		3.50	
	97.00		3.34	
Barley......................	75.00	64.5	2.57	2.21
	62.00		2.13	
	53.00		1.82	
	68.00		2.34	
Rye.........................	92.00	94.00	3.16	3.22
	97.00		3.34	
	87.00		2.99	
	99.00		3.40	
Sugar beets..................	32.00	31.00	1.10	1.07
	26.00		0.90	
	31.00		1.07	
	35.00		1.20	
Red clover..................	50.00	43.00	1.72	1.48
	48.00		1.65	
	36.00		1.24	
	38.00		1.31	

TABLE 3—*Continued*

CROP GROWN	PARTS PER MILLION	AVERAGE	GRAMS	AVERAGE
Vetch........................	50.00	53.00	1.72	1.81
	52.00		1.79	
	54.00		1.86	
	55.00		1.89	
Soybeans......................	44.00	39.50	1.51	1.35
	37.00		1.27	
	37.00		1.27	
	40.00		1.37	
Field peas....................	86.00	97.0	2.96	3.34
	89.00		3.06	
	134.00		4.60	
	80.00		2.75	

discrepancy in the amount of nitrate formed in the cropped soils plus that removed by the crop can possibly be explained by one of two suppositions: either nitrification is inhibited by the growth of the crop or nitrification proceeds as rapidly as in the fallow check but some nitrogen is wasted incidental to crop growth. The latter supposition seems to be borne out by table 2 and figure 1, which show a distinct loss of total nitrogen from the soil due to crop growth in every instance except where soybeans were grown. In that case there is no means of knowing if some loss of nitrogen did occur, with nitrogen fixation. Since the fallow check lost considerable total nitrogen, as shown in table 2 and figure 1, possibly more nitrate was formed here than is shown in table 3 and figure 2; in which case the deficit in nitrate recovered from the cropped soils would be greater than is shown.

In table 4 is shown the comparative rate of nitrification in the various soils expressed as the gain in nitrate produced during two weeks' incubation at 28°C. of 100 gm. of soil with 0.2 per cent peptone. These results show some evidence of nitrification having been affected by the residual influence of crops. Nitrification following corn, wheat, beets, soybeans and field peas shows evidence of inhibition, while that following barley and vetch is evidence of stimulation. Following the rest of the crops, millet, kafir corn, oats, rye and clover, the residual influence apparently is neutral.

In 1915 the same general plan was followed except that the experiment was carried in triplicate in three parallel series on as many different types of soils. First, a virgin soil was selected from near Riverside, California. It is a coarse, gravelly loam. The second soil was selected from near Manhattan, Kansas. This is a heavy, black silt loam. The third soil was a clay loam from the Government Arlington Experiment Farm. Chemical analyses of
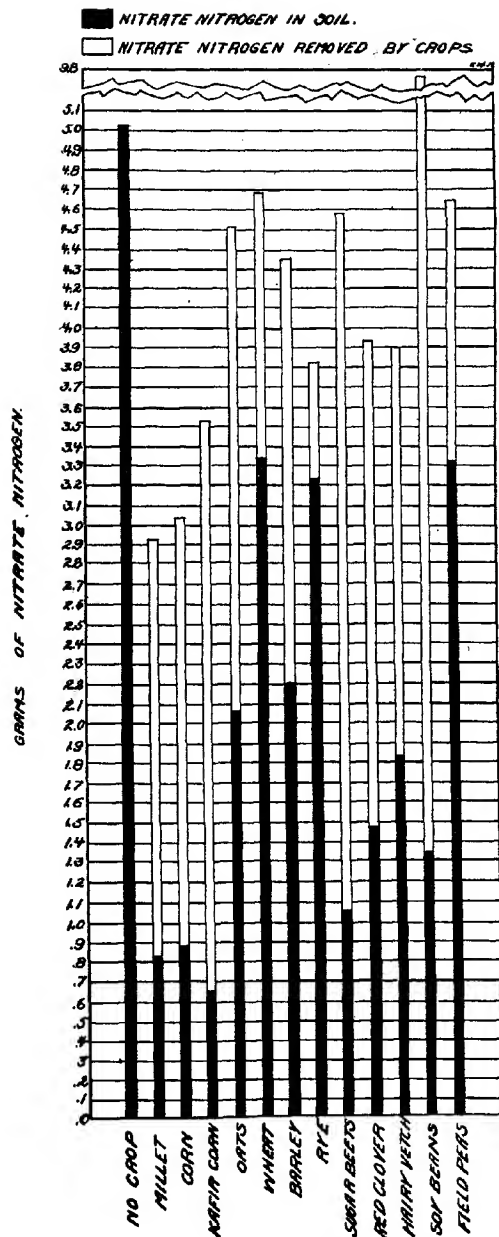
FIG. 2. NITRATE NITROGEN FOUND IN THE SOIL AFTER HARVEST AND THAT REMOVED BY THE CROPS

TABLE 4

Nitrate nitrogen produced during two weeks' incubation of peptone in soil after harvest

CROP GROWN	INCREASE	AVERAGE INCREASE
	p.p.m.	*p.p.m.*
Fallow check..	185.00 202.00	195.00
Millet...	183.00 206.00 205.00 187.00	195.00
Corn..	178.00 166.00 182.00 187.00	178.00
Kafir corn..	164.00 203.00 205.00 194.00	191.50
Oats..	193.00 226.00 178.00	199.00
Wheat..	174.00 189.00 162.00 197.00	181.5
Barley..	209.00 229.00 229.00 214.00	220.00
Rye...	225.00 178.00 175.00 189.00	191.50
Sugar beets...	175.00 176.00 185.00 208.00	186.00
Red clover..	214.00 189.00 181.00 206.00	197.50

TABLE 4—*Continued*

CROP GROWN	INCREASE	AVERAGE INCREASE
	p.p.m.	*p.p.m.*
Vetch	208.00	201.00
	188.00	
	200.00	
	211.00	
Soybeans	190.00	173.50
	191.00	
	162.00	
	162.00	
Field peas	202.00	175.50
	153.00	
	145.00	
	202.00	

these soils made by the Bureau of Soils of the United States Department of Agriculture are given as follows:

	K_2O	CaO	MgO	P_2O_5
	per cent	*per cent*	*per cent*	*per cent*
California soil	1.91	3.13	1.45	0.21
Kansas soil	2.18	1.13	0.86	0.16
Virginia soil	1.72	1.98	0.72	0.19

Since the Kansas and Virginia soils tested acid to litmus they were limed to give a neutral reaction previous to weighing into buckets. The crops grown during this year were oats, barley, kafir corn, crimson clover, soybeans, purple vetch and cowpeas. No attempt will be made to draw any comparisons between these three soils from the standpoint of determining their relative fertility or productiveness, this not being the purpose of the experiment.

The crop yields in grams of dry matter are shown in table 5. In tables 6, 7 and 8 are shown in grams the nitrogen per bucket recovered in the soils after removal of the crops and that recovered in the crops; also the nitrogen in the original soil and that in the samples kept fallow.

In the California soil, as in results already described, considerable nitrogen was lost from the fallow soil. From soil growing oats, kafir corn and vetch, not as much nitrogen was recovered from both soil and crops as was found in the fallow check. Slightly more nitrogen was recovered from barley and clover soils than from the fallow check. The soils under soybeans and cowpeas contained more nitrogen after harvesting the crops than any of the other soils, but somewhat less than the dry check, while the nitrogen found in the

plants was considerably more than was accounted for in that removed from the soil, indicating active fixation.

In the Kansas soil also nitrogen was lost from the fallow. Oats and clover did not remove as much nitrogen from the soil as the fallow. Not as much nitrogen was recovered in the soil and plants in the case of barley and kafir corn as was found in the fallow. Vetch fixed some nitrogen and there was slightly more nitrogen found in the soil than in the dry check, but the amount

TABLE 5

Dry weights of crops harvested

CROP GROWN	CALIFORNIA SOIL		KANSAS SOIL		VIRGINIA SOIL	
	Dry matter	Average	Dry matter	Average	Dry matter	Average
	gm.	gm.	gm.	gm.	gm.	gm.
Oats.................	36.0	36.5	38.0	30.2	13.5	12.7
	37.0		14.5		14.5	
	36.5		38.0		10.0	
Barley.................	35.5	29.5	16.5	26.0	11.5	10.8
	25.5		32.0		10.0	
	27.5		29.5		11.0	
Kafir corn..............	63.0	65.7	102.0	99.0	121.0	117.7
	74.0		128.0		112.0	
	60.0		67.0		120.0	
Crimson clover..........	31.5	29.3	35.0	27.2	11.0	12.7
	22.5		23.0		17.0	
	20.5		23.5		10.0	
Soybeans...............	132.0	127.3	71.0	59.8	61.0	63.7
	132.0		45.0		59.0	
	118.0		63.5		71.0	
Vetch.................	19.0	13.8	7.5	7.2	2.5	7.7
	12.0		6.5		8.5	
	10.5		7.5		12.0	
Cowpeas...............	121.0	130.7	125.0	101.2	134.5	159.5
	160.0		83.5		167.5	
	111.0		95.0		176.5	

was easily within the range of analytical error. There was some fixation of nitrogen with cowpeas and practically as much nitrogen was found in the soil as in the fallow soil.

In the Virginia soil there was again considerable loss of nitrogen from the fallow. More nitrogen was recovered from oats and soil than from the fallow. Under barley, clover and vetch more nitrogen was recovered in the soil than in the fallow; but the total in the soil and the plants did not equal that in

the dry check. Not as much nitrogen was recovered from kafir corn and soil as was found in the fallow. Soybeans removed a little more nitrogen than the fallow but no total fixation was indicated. Cowpeas did not remove as much nitrogen as the fallow and considerable total fixation was shown from soil and crop over that present in the dry check.

TABLE 6

Total nitrogen remaining in the soil and recovered in crops after harvest

(California soil)

CROPS GROWN	SOIL		CROPS		TOTAL NITROGEN RECOVERED
	Nitrogen	Average	Nitrogen	Average	
	gm.	gm.	gm.	gm.	gm.
Dry check..........	18.3	18.3			18.30
Fallow check.......	16.3	16.3			16.30
Oats.............	15.3	15.3	0.35	0.34	15.64
	15.3		0.34		
			0.33		
Barley...........	16.5	16.4	0.33	0.29	16.69
	15.3		0.26		
	17.4		0.29		
Kafir corn........	15.8	15.4	0.36	0.38	15.78
	15.3		0.43		
	15.0		0.36		
Crimson clover.....	15.8	16.3	0.80	0.58	16.88
	15.7		0.50		
	17.5		0.43		
Soybeans..........	16.9	17.4	3.66	3.66	21.06
	17.6		3.76		
	17.6		3.56		
Vetch.............	15.8	15.9	0.52	0.35	16.25
	16.3		.0.27		
	15.7		0.25		
Cowpeas..........	17.6	17.6	2.81	3.01	20.61
	17.6		3.40		
	17.6		2.82		

To sum up more briefly: In all three types of soil there was a loss of nitrogen under fallow. There was a greater total loss than under fallow in the case of oats, kafir corn, and vetch in the California soil; barley and kafir corn in the Kansas soil and kafir corn in the Virginia soil. Not as much nitrogen was removed from the soil as under fallow in the cases of barley, soybeans

and cowpeas in the California soil, oats, clover, vetch and cowpeas in the Kansas soil, and oats, barley, clover, vetch and cowpeas in the Virginia soil. There was more total nitrogen recovered in the soil and crops than was originally present in the cases of soybeans and cowpeas in the California soil, vetch and cowpeas in the Kansas soil, and cowpeas in the Virginia soil.

TABLE 7

Total nitrogen remaining in the soil and recovered in crops after harvest

(Kansas soil)

CROPS GROWN	SOIL		CROPS		TOTAL NITROGEN RECOVERED
	'Nitrogen	Average	Nitrogen	Average	
	gm.	gm.	gm.	gm.	gm.
Dry check..........	51.5	51.5			51.50
Fallow check.......	50.4	50.4			50.40
Oats.............	50.8	50.6	0.78	0.55	51.15
	51.2		0.24		
	49.7		0.62		
Barley...........	50.4	49.6	0.36	0.53	50.13
	49.0		0.63		
	49.4		0.60		
Kafir corn........	48.5	49.1	1.18	0.94	50.04
	49.4		1.05		
	49.5		0.58		
Crimson clover.....	51.0	50.5	0.87	0.70	51.20
	50.0		0.70		
	50.4		0.53		
Soybeans..........	49.0	49.1	1.91	1.67	50.77
	50.0		1.32		
	48.4		1.79		
Vetch.............	52.4	51.7	0.20	0.21	51.91
	51.3		0.19		
	51.4		0.23		
Cowpeas..........	51.0	50.5	2.74	2.17	52.67
	50.2		1.88		
	50.4		1.94		

In figure 3 is illustrated, as in figure 2, the nitrate nitrogen recovered in the soils after harvest, together with that found in the associated crop, assuming that the nitrogen found in the crop was removed from the soil as nitrate. Here again is shown several instances where either not as much nitrate was formed as that found in the fallow checks, or as much or more was formed and

subsequently lost. In the California soil under oats, barley and kafir corn, not as much nitrate was recovered as in the fallow. Under clover and vetch somewhat more nitrate was recovered than was found in the fallow, although figure 3 shows a distinct loss in total nitrogen in both these instances. Under soybeans and cowpeas there was considerable nitrogen fixation as shown in

TABLE 8

Total nitrogen remaining in soil and recovered in crops after harvest

(Virginia soil)

CROPS GROWN	SOIL		CROPS		TOTAL NITROGEN RECOVERED
	Nitrogen	Average	Nitrogen	Average	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Dry check..........	48.2	48.2			48.2
Fallow check.......	46.3	46.3			46.3
Oats..............	46.5	46.6	0.34	0.30	46.90
	46.3		0.33		
	47.0		0.24		
Barley...........	47.8	47.8	0.31	0.31	48.11
	45.8		0.29		
	49.8		0.34		
Kafir corn........	45.1	45.0	1.15	1.17	46.17
	45.4		1.32		
	44.5		1.04		
Crimson clover.....	46.5	47.2	0.34	0.43	47.63
	47.8		0.58		
	47.2		0.36		
Soybeans..........	46.3	45.8	1.71	1.88	47.68
	45.1		1.86		
	46.0		2.06		
Purple vetch.......	47.1	47.1	0.07	0.25	47.35
	47.2		0.29		
	47.0		0.40		
Cowpeas..........	46.3	47.0	4.60	4.90	51.90
	47.1		5.00		
	47.5		5.10		

figure 3, therefore, it is uncertain how much of this was obtained from the soil as nitrate. In the Kansas soil there was a deficit in the amount of nitrate recovered as compared with that found in the fallow in every case except that of soybeans. In this instance there was also a fixation of nitrogen. In the Virginia soil as in the Kansas soil there is again a deficit in the amounts of
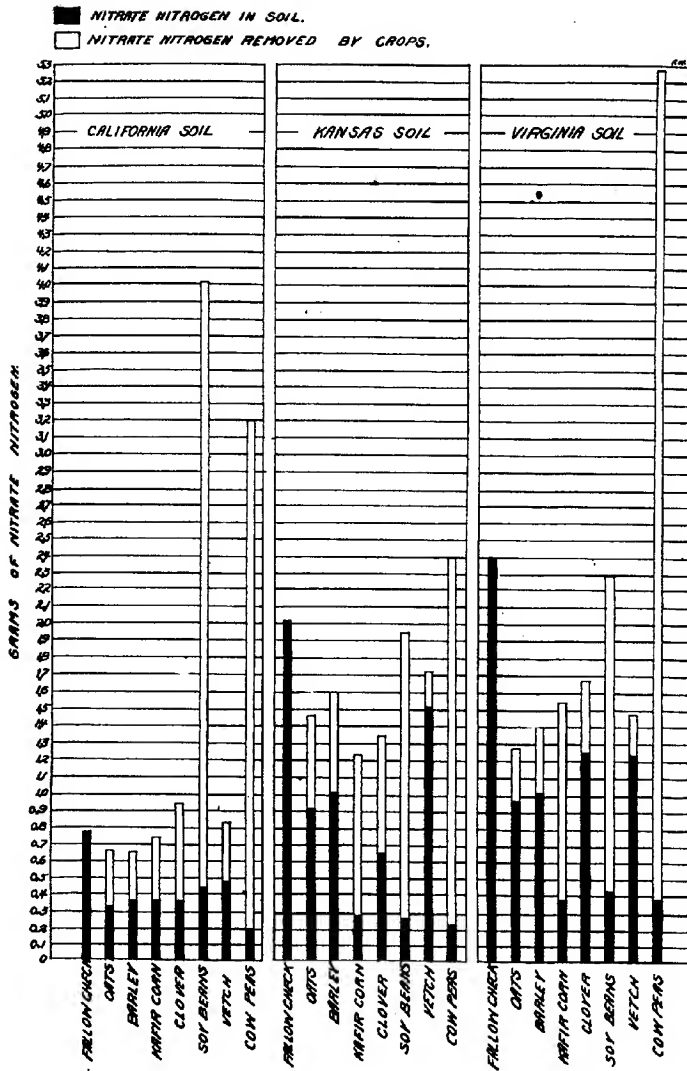
FIG. 3. NITRATE NITROGEN FOUND IN THE CALIFORNIA, KANSAS AND VIRGINIA SOILS AFTER
HARVEST AND THAT REMOVED BY THE CROPS

nitrate recovered in all cases except under cowpeas, where again there was a fixation of nitrogen.   Table 9, which shows the comparative nitrifying power or the gain in nitrogen produced during two weeks' incubation of ammonium sulfate (0.139 per cent) with soil at 28°C., seems to indicate that there is more or less crop influence on the formation of nitrate in the soil, thus possibly explaining the results shown in figure 4.

TABLE 9

Nitrate nitrogen produced during two weeks' incubation of ammonium sulfate in soil after harvest

CROP GROWN	CALIFORNIA SOIL		KANSAS SOIL		VIRGINIA SOIL	
	Increase	Average	Increase	Average	Increase	Average
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Fallow check.............	71.4	71.4	15.2	15.2	261.7	261.7
Oats..................	78.9	71.1	23.6	25.1	310.0	300.3
	63.4		31.3		299.0	
			20.4		297.0	
Barley................	68.1	63.2	7.4	12.0	266.5	271.6
	58.8		13.0		267.3	
	62.8		15.7		281.0	
Kafir corn.............	51.5	53.3	12.7	8.1	270.1	273.7
	55.3		7.5		275.1	
	53.0		4.2		275.8	
Crimson clover..........	65.9	66.3	19.5	18.8	304.0	300.2
	65.0		20.3		292.0	
	68.1		16.6		304.5	
Soybeans..............	64.2	60.9	16.6	18.8	283.0	266.4
	53.3		21.7		249.0	
	65.1		18.1			
Vetch.................	71.3	70.7	16.8	18.4	293.0	291.8
	70.9		17.4		299.3	
	70.0		21.0		283.0	
Cowpeas...............	61.0	55.4	14.3	11.1	240.8	233.5
	53.5		15.3		237.8	
	51.7		3.7		221.8	

In 1916 a series of experiments were inaugurated in which the California, Kansas and Virginia soils were used as before; however, in this series the same buckets were cropped for three years and the crops removed without the addition of plant-food in any form.   Each season after harvest the buckets were covered to shed rain, and left undisturbed for the winter. The crops grown in each type of soil were as follows: corn, wheat, oats, cotton, soybeans

and cowpeas. In addition, the following rotations were grown in each type of soil: corn, wheat, oats; corn, oats, wheat; soybeans, wheat, oats; soybeans, oats, wheat; and cowpeas, soybeans, cowpeas.

TABLE 10

Dry-weight yields of crops in three-year rotation

(California soil)

	1916			1917			1918	
Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Corn	81.0 98.0 97.0 86.0	90.5	Corn	71.0 84.0 72.0 66.0	73.2	Corn	42.0 50.0 45.0 47.0	46.0
Corn	85.0 98.0 95.0 100.0	94.5	Wheat	37.5 27.0 35.5 39.5	34.9	Oats	31.0 28.0 28.0 32.0	29.7
Corn	96.0 85.0 97.5 83.5	93.0	Oats	43.0 46.5 47.5 50.0	46.7	Wheat	20.0 20.0 22.0 23.0	21.2
Wheat	28.5 33.0 31.0 36.0	32.1	Wheat	45.5 44.0 44.5 42.0	44.0	Wheat	22.0 23.0 27.0 22.0	23.5
Oats	39.0 44.0 44.5 47.0	43.6	Oats	57.0 56.0 53.0 56.5	55.6	Oats	27.0 27.0 27.0 28.0	27.2
Cotton	71.0 69.0 82.0 86.0	77.0	Cotton	39.0 48.0 30.0 32.0	37.2	Cotton	13.0 10.0 12.0 11.0	11.7
Soybeans	450.0 360.0 341.0 362.0	378.2	Soybeans	185.0 113.0 185.0 73.0	139.0	Soybeans	40.0 45.0 63.0 27.0	43.7
Soybeans	326.0 342.0 333.0 382.0	345.8	Wheat	41.5 51.5 37.0 44.5	43.6	Oats	33.0 32.0 33.0 39.0	34.2

TABLE 10—Continued

1916			1917			1918		
Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average
	gm.	gm.		gm.	gm.		gm.	gm.
Soybeans	289.0 335.0 434.0 336.0	348.5	Oats	62.5 70.0 63.5 69.5	66.4	Wheat	20.0 23.0 25.0 27.0	23.7
Cowpeas	205.0 214.0 187.0 219.0	206.2	Cowpeas	162.0 125.0 105.0 147.0	134.7	Cowpeas	89.0 115.0 87.0 97.0	97.5
Cowpeas	229.0 352.0 197.5 218.5	249.2	Soybeans	122.0 172.0 180.0 158.0	158.0	Cowpeas	112.0 90.0 105.0 93.0	100.0

FIG. 4. TOTAL NITROGEN REMAINING IN THE CALIFORNIA SOIL EACH YEAR AND THAT RECOVERED IN THE CROPS

TABLE 11

Dry-weight yields of crops in three-year rotation

(Kansas soil)

1916			1917			1918		
Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Corn	168.0 165.0 167.0 140.0	160.0	Corn	85.0 88.0 89.0 83.5	86.4	Corn	48.0 52.0 71.0 53.0	56.0
Corn	157.5 171.5 187.0 140.5	164.1	Wheat	49.0 43.0 54.0 38.0	46.0	Oats	36.0 25.0 31.0 33.0	31.2
Corn	165.0 173.0 161.0 154.0	163.2	Oats	50.0 52.0 46.0 60.0	52.0	Wheat	12.0 20.0 26.0 22.0	20.0
Wheat	53.0 55.0 67.0 52.5	56.9	Wheat	34.0 46.0 48.5 28.0	39.1	Wheat	26.0 35.0 28.0 35.0	36.0
Oats	85.0 83.0 100.0 89.0	89.2	Oats	56 0 53.0 54.0 63.0	56.5	Oats	39.0 16.0 27.0 37.0	27.7
Cotton	142.0 178.0 136.0 128.0	146.0	Cotton	28.0 46.0 28.0 45.0	36.7	Cotton	32.0 37.0 29.0 50.0	37.0
Soybeans	230.0 208.0 196.0 231.0	216.2	Soybeans	67.0 75.0 97.0 68.0	76.7	Soybeans	35.0 65.0 65.0 47.0	53.0
Soybeans	230.0 208.0 196.0 231.0	216.2	Soybeans	67.0 75.0 97.0 68.0	76.7	Soybeans	35.0 65.0 65.0 47.0	53.0

TABLE 11—*Continued*

1916			1917			1918		
Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Soybeans	209.0	186.0	Wheat	37.5	39.9	Oats	27.0	24.7
	195.0			36.0			25.0	
	209.0			39.0			27.0	
	131.0			47.0			20.0	
Soybeans	285.0	211.7	Oats	40.0	44.0	Wheat	11.0	18.5
	224.0			40.0			19.0	
	147.0			42.0			24.0	•
	191.0			54.0			20.0	
Cowpeas	130.0	128.4	Cowpeas	73.0	59.7	Cowpeas	20.0	47.7
	119.5			42.0			65.0	
	138.0			59.0			64.0	
	126.0			65.0			42.0	
Cowpeas	148.0	167.7	Soybeans	68.0	88.5	Cowpeas	32.0	34.0
	192.0			96.0			30.0	
	142.0			103.0			34.0	
	189.0			87.0			40.0	

Tables 10, 11 and 12 show the dry weights of the crops harvested each year. As might be expected, the yield each succeeding year rapidly decreased.

Tables 13, 14 and 15, and figures 4, 5 and 6 show the amounts of total nitrogen remaining in the soil and that recovered in the crops each year. The results shown in the figures represent averages.

In the California soil the results were somewhat different from those found in the other soils in that there was a general tendency in some samples toward an increase in nitrogen each year, while in others there was a decrease the first two years followed by a considerable increase the third year in all samples. In the other two types of soils there was a general decrease each year in all cases.

During this period in the California soil the greatest increase occurred where the same crops were grown in succession, this increase being greater than either under fallow or under rotation. The increase under fallow was less than under rotation. Under soybeans and cowpeas the third year there was more nitrogen found than was in the soil at the beginning of the experiment, as represented by the dry check. Here we have the results of a curious combination of phenomena.

With the exception of the legumes the first two years there was a considerable loss in nitrogen, that is, not as much nitrogen was recovered in the crops as was removed from the soil. Under corn the second year the total loss

TABLE 12

Dry-weight yields of crops in three-year rotation

(Virginia soil)

1916			1917			1918		
Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Corn	319.0	322.2	Corn	85.0	86.4	Corn	80.0	77.0
	296.0			88.0			74.0	
	325.0			89.0			79.0	
	349.0			83.5			75.0	
Corn	304.0	325.2	Wheat	49.0	46.0	Oats	36.0	36.7
	347.0			43.0			39.0	
	296.0			54.0			37.0	
	354.0			38.0			35.0	
Corn	361.0	343.0	Oats	50.0	52.0	Wheat	12.0	16.0
	302.0			52.0			11.0	
	324.5			46.0			17.0	
	385.0			60.0			24.0	
Wheat	65.0	63.0	Wheat	34.0	39.1	Wheat	30.0	26.5
	63.0			46.0			21.0	
	65.5			48.5			25.0	
	58.5			28.0			30.0	
Oats	144.0	143.25	Oats	56.0	56.5	Oats	36.0	27.7
	157.0			53.0			24.0	
	128.0						26.0	
	144.0						33.0	
Cotton	269.0	286.0	Cotton	28.0	36.7	Cotton	25.0	24.7
	343.0			46.0			14.0	
	250.0			28.0			30.0	
	282.0			45.0			30.0	
Soybeans	41.8	369.5	Soybeans	67.0	76.7	Soybeans	63.0	74.2
	367.0			75.0			84.0	
	334.0			97.0			67.0	
	359.0			68.0			83.0	
Soybeans	418.0	369.5	Soybeans	67.0	76.7	Soybeans	63.0	74.2
	367.0			75.0			84.0	
	334.0			97.0			67.0	
	359.0			68.0			83.0	

TABLE 12—*Continued*

1916			1917			1918		
Crop grown	Dry weight of crop	Average	. Crop grown	Dry weight of crop	Average	Crop grown	Dry weight of crop	Average
	gm.	*gm.*		*gm*	*gm.*		*gm.*	*gm.*
Soybeans	354.0 343.0 370.0 370.0	359.2	Oats	37.5 36.0 39.0 47.0	39.9	Wheat	91.0 32.0 38.0	53.7
Soybeans	345.0 329.0 407.0 310.0	347.7	Oats	40.0 40.0 42.0 54.0	44.0	Wheat	22.0 24.0 29.0 22.0	26.7
Cowpeas	251.0 172.0 172.0 220.0	203.7	Cowpeas	73.0 42.0 59.0 65.0	59.7	Cowpeas	15.0 23.0 27.0	21.7
Cowpeas	147.0 218.0 257.0 227.0	212.2	Soybeans	68.0 96.0 103.0 87.0	88.5	Cowpeas	41.0 41.0 29.0 42.0	38.2

amounted to 518 gm. per average bucket. In the case of soybeans and cowpeas, there was an increase in nitrogen the first year, due evidently to symbiotic fixation. This increase was greater under soybeans than under cowpeas. The second year there was still enough nitrogen recovered in the crops almost to make the total of that in the soils and that recovered in the crops equal to that in the dry check. The gain in nitrogen noted the third year in all samples is evidently due to fixation in the soil through biological action. The processes apparently at work more or less simultaneously were denitrification, non-symbiotic and symbiotic nitrogen fixation. Through denitrification there was a total loss of a comparatively large amount of nitrogen from the soil in all samples the first year, with the exception of those under legumes. In some cases during the second year this process still predominated. In other cases including the fallow, free fixation of nitrogen apparently commenced to predominate. The third year this latter process caused the recovery of nearly all the nitrogen that was lost the first year in most cases. Under soybeans and cowpeas the additional process of symbiotic nitrogen fixation was also at work. At the end of the third year there was more nitrogen in the soils under these crops than at the beginning of the experiment, while the total amount of nitrogen for the three years contained in the crops was a clear gain.

In the Kansas soil under the non-legumes with but one exception there was a successive loss of nitrogen each year. In general this loss was progressive,

TABLE 13

Total nitrogen in three-year rotation

(California soil)

Crop grown	1916				1917					1918				
	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average	Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in soil	Average	Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average
	gm.	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*
Corn	• 14.8	14.5	0.31	0.36	Corn	11.6	13.8	0.35	0.39	Corn	19.2	18.6	0.23	0.90
	15.3		0.39			14.1		0.37			17.9		0.25	
	14.4		0.37			14.8		0.48			19.6		0.23	
	13.6		0.36			14.6		0.38			17.6		0.20	
Corn	15.0	15.4	0.36	0.39	Wheat	15.0	15.3	0.38	0.38	Oats	17.9	17.8	0.28	0.26
	16.4		0.42			15.9		0.30			17.9		0.26	
	15.3		0.38			14.8		0.46			17.9		0.27	
	14.8		0.40			15.7		0.40			17.4		0.33	
Corn	16.6	15.3	0.40	0.41	Oats	16.4	15.6	0.35	0.38	Wheat	18.9	18.7	0.20	0.21
	15.2		0.40			15.3		0.39			19.1		0.22	
	14.8		0.42			15.1		0.41			18.5		0.22	
	14.6		0.41			15.7		0.38			18.5		0.22	
Wheat	15.2	15.1	0.18	0.21	Wheat	15.1	14.5	0.48	0.49	Wheat	17.2	18.2	0.22	0.25
	15.2		0.22			13.4		0.47			17.4		0.23	
	14.4		0.20			14.0		0.48			20.4		0.26	
	15.5		0.24			15.5		0.53			17.9		0.28	
Oats	15.1	15.0	0.46	0.47	Oats	16.0	14.9	0.48	0.45	Oats	18.4	18.3	0.27	0.29
	14.5		0.49			14.0		0.46			18.6		0.31	
	15.3		0.45			14.2		0.39			17.9		0.28	
	15.1		0.47			15.3		0.47			18.3		0.31	

Crop					Crop					Crop				
Cotton	14.0 15.7 14.2 14.2	14.5	0.61 0.61 0.68 0.73	0.66	Cotton	14.4 15.3 17.9 14.6	15.5	0.41 0.32 0.30 0.27	0.32	Cotton	18.7 20.0 18.2 19.1	19.0	0.12 0.13 0.12 0.12	0.12
Soybeans	16.2 17.0 16.2 18.1	16.9	10.5 8.7 8.2 9.4	9.21	Soybeans	16.3 15.1 14.6 15.7	15.4	5.65 3.18 5.00 1.98	3.95	Soybeans	19.5 20.8 19.8 19.8	20.0	1.06 1.42 1.84 0.64	1.24
Soybeans	17.9 14.7 16.8 15.2	16.1	7.9 7.9 8.6 9.2	8.40	Wheat	14.9 16.3 16.9 16.5	16.1	0.64 0.70 0.57 0.58	0.62	Oats	20.0 18.7 18.9 18.3	19.0	0.45 0.36 0.38 0.45	0.41
Soybeans	15.7 16.1 16.1 16.1	16.0	7.7 8.0 10.6 8.8	8.76	Oats	15.5 16.8 16.6 15.0	16.0	0.64 0.82 0.64 0.81	0.73	Wheat	19.3 18.3 17.6 17.4	18.1	0.28 0.33 0.38 0.33	0.33
Cowpeas	17.2 16.2 17.2 15.7	16.6	3.8 4.6 3.8 4.9	4.26	Cowpeas	16.2 16.8 18.0 16.5	16.9	3.42 2.84 2.26 2.86	2.84	Cowpeas	22.5 20.6 23.1 20.8	21.7	2.47 1.96 2.51	2.31
Cowpeas	17.0 19.6 16.1	17.6	4.0 6.5 4.5 4.8	4.95	Soybeans	16.5 18.8 16.3 16.6	17.0	4.20 5.02 4.65 3.77	4.41	Cowpeas	18.7 19.5 19.9 19.3	19.3	3.15 2.73 2.77 2.50	2.79
Fallow check	15.5 18.5 16.1 14.2	16.1			Fallow check	17.2 16.0 15.8 16.8	16.4			Fallow check	19.5 20.1 19.1 19.1	19.4		
										Dry check	19.6			

TABLE 14

Total nitrogen in three-year rotation

(Kansas soil)

1916					1917					1918				
Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average	Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average	Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average
	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.
Corn	45.5	46.1	0.83	0.82	Corn	44.0	43.6	0.61	0.63	Corn	42.6	43.0	0.35	0.44
	45.7		0.86			43.2		0.61			42.9		0.40	
	46.6		0.82			42.0		0.67			42.9		0.62	
	46.5		0.76			44.2		0.62			43.5		0.39	
Corn	45.5	44.8	0.74	0.81	Wheat	44.2	43.4	0.86	0.77	Oats	42.5	41.0	0.63	0.53
	43.2		0.83			43.2		0.66			39.5		0.43	
	45.5		0.95			43.7		0.90			41.0		0.52	
	45.0		0.73			42.7		0.68			41.1		0.53	
Corn	45.0	45.6	0.83	0.85	Oats	43.3	44.4	0.86	0.87	Wheat	39.9	40.8	0.20	0.32
	45.0		0.94			44.7		0.66			41.3		0.33	
	45.8		0.79			44.1		0.74			41.0		0.40	
	46.7		0.85			45.5		0.83			41.2		0.35	
Wheat	42.6	43.1	0.98	1.02	Wheat	43.8	44.3	0.56	0.67	Wheat	42.5	42.5	0.42	0.48
	43.3		0.98			44.7		0.84			42.2		0.55	
	43.0		1.18			44.2		0.86			43.8		0.43	
	43.2		0.94			44.6		0.43			41.5		0.54	
Oats	43.5	44.4	1.26	1.24	Oats	43.3	43.8	0.81	0.85	Oats	42.7	42.9	0.58	0.45
	44.7		1.16			46.1		0.81			44.1		0.28	
	44.7		1.28			42.6		0.80			43.9		0.42	
	44.7		1.28			43.2		0.98			41.1		0.52	

Crop	Values	Avg	Values	Avg
Cotton	45.0, 46.1, 44.0, 45.2	45.1	1.46, 1.94, 1.28, 1.44	1.53
Soybeans	46.5, 46.4, 46.2, 45.5	46.2	4.95, 4.46, 4.05, 4.66	4.53
Soybeans	45.0, 46.1, 45.4, 46.0	45.6	4.21, 4.61, 4.56, 2.60	3.99
Soybeans	45.0, 45.0, 46.0, 47.6	45.9	5.90, 4.66, 3.35, 4.10	4.50
Cowpeas	43.2, 47.1, 46.6, 45.0	45.5	2.67, 1.90, 2.60, 2.53	2.42
Cowpeas	44.5, 44.0, 48.0, 44.8	45.3	2.57, 2.35, 2.82, 3.35	3.02
Fallow check	46.6, 47.5, 46.4, 47.0	46.9		

Crop	Values	Avg	Values	Avg
Cotton	44.2, 43.8, 43.2, 44.7	44.0	0.44, 0.63, 0.57, 0.58	0.55
Soybeans	43.3, 44.7, 46.1, 46.3	45.1	1.52, 1.66, 2.00, 1.54	1.68
Wheat	44.1, 44.2, 46.7, 44.7	44.9	0.58, 0.54, 0.61, 0.70	0.61
Oats	45.3, 45.2, 45.2, 45.6	45.3	0.61, 0.61, 0.61, 0.79	0.65
Cowpeas	45.3, 47.7, 44.6, 45.0	45.6	1.42, 0.85, 1.23, 1.23	1.18
Soybeans	45.4, 44.7, 43.8, 45.2	44.8	1.60, 1.82, 1.96, 1.94	1.83
Fallow check	44.6, 46.0, 45.2, 45.7	45.4		

Crop	Values	Avg	Values	Avg
Cotton	42.4, 42.0, 42.8, 43.8	42.7	0.57, 0.56, 0.52, 0.64	0.57
Soybeans	42.8, 44.0, 43.6, 42.8	43.3	0.86, 1.23, 1.36, 0.99	1.11
Oats	43.1, 41.6, 41.5, 42.6	42.2	0.42, 0.44, 0.43, 0.36	0.41
Wheat	42.8, 42.2, 43.0, 42.6	42.6	0.20, 0.28, 0.36, 0.32	0.29
Cowpeas	43.3, 45.0, 45.0, 43.8	44.3	0.38, 1.23, 1.38, 0.73	0.93
Cowpeas	42.6, 43.7, 42.2, 43.5	43.0	0.68, 0.59, 0.65, 0.79	0.68
Fallow check	43.8, 45.5, 45.4, 45.6	45.1		
Dry check	47.0			

TABLE 15

Total nitrogen in three-year rotation

(Virginia soil)

	1916					1917					1918			
Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average	Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average	Crop grown	Nitrogen remaining in soil	Average	Nitrogen recovered in crop	Average
	gm.	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*
Corn	43.3	43.2	4.17	4.04	Corn	40.4	39.4	2.67	2.24	Corn	38.6	38.6	0.81	0.75
	42.6		3.97			39.0		2.38			38.9		0.78	
	43.5		3.96			39.2		2.12			39.2		0.65	
	43.5		4.06			39.0		1.78			37.7		0.77	
Corn	43.7	43.5	4.36	3.80	Wheat	39.8	40.1	1.11	1.32	Oats	39.6	39.4	0.64	0.67
	44.7		3.86			40.5		1.66			39.4		0.72	
	43.3		3.64			40.6		1.34			39.3		0.68	
	42.3		3.96			39.6		1.18			39.3		0.66	
Corn	44.2	43.5	3.40	2.90	Oats	40.4	39.9	1.91	1.87	Wheat	39.9	38.7	0.19	0.25
	43.5		3.37			39.1		1.95			38.6		0.18	
	44.0		4.44			40.5		2.02			39.3		0.25	
	42.3		4.40			39.7		1.60			37.2		0.37	
Wheat	46.5	46.1	1.30	1.37	Wheat	42.6	42.6	1.39	1.34	Wheat	42.0	41.1	0.52	0.47
	45.2		1.42			42.2		1.02			40.9		0.38	
	46.4		1.41			43.6		1.39			41.3		0.44	
	46.3		1.35			42.0		1.56			40.3		0.53	
Oats	44.0	43.8	3.07	3.03	Oats	40.7	40.4	2.08	2.05	Oats	37.8	38.1	0.60	0.52
	43.3		3.35			40.8		1.93			37.7		0.41	
	44.7		2.65			39.3		2.24			38.2		0.48	
	43.2		3.05			40.7		1.95			38.6		0.61	

					Avg					Avg						Avg					Avg						Avg					Avg
Cotton	42.2	42.4	44.8	43.2	43.1	4.55	6.03	4.29	4.71	4.89	Cotton	38.8	38.4	38.1	40.0	38.9	1.94	1.66	1.97	1.81	1.84	Cotton	37.6	38.9	38.4	37.9	38.2	0.71	0.46	0.80	0.85	0.70
Soybeans	44.7	43.7	44.2	44.1	44.2	11.78	10.00	9.05	9.10	9.98	Soybeans	41.3	40.5	42.3	39.8	41.3	6.14	5.18	5.94	6.67	5.98	Soybeans	39.3	40.1	38.2	39.3	39.2	1.55	2.20	1.76	2.15	1.91
Soybeans	44.4	41.2	43.2	43.4	43.8	9.10	7.65	9.30	9.10	8.79	Wheat	40.8	41.7	40.8	39.8	40.8	0.86	1.18	1.06	1.10	1.05	Oats	38.9	38.2	38.6	38.9	38.6	1.77		0.60	0.75	0.78
Soybeans	42.3	42.6	42.7	42.7	42.6	8.85	8.75	11.05	7.60	9.06	Oats	40.4	41.7	41.0	41.7	41.2	1.90	1.74	2.14	1.78	1.89	Wheat	38.2	38.6	37.3	38.9	38.2	0.39	0.58	0.50	0.41	0.47
Cowpeas	42.9	42.8	46.6	46.2	44.6	5.47	4.77	4.07	4.49	4.70	Cowpeas	40.3	40.5	40.5	40.2	40.3	3.23	3.44	3.14	3.07	3.22	Cowpeas	42.2	40.3	41.3	40.3	41.0	0.44	0.55	0.88		0.62
Cowpeas	42.5	45.1	45.3	42.8	43.9	3.80	5.20	5.70	4.72	4.86	Soybeans	39.8	39.3	41.3	39.3	39.9	4.16	5.18	4.52	5.77	4.91	Cowpeas	40.8	39.5	31.5	40.6	38.8	0.86	0.96	0.68	0.81	0.83
Fallow check	48.6	47.6	48.3	47.5	48.0						Fallow	47.8	46.7	46.5	46.4	46.8						Fallow	43.6	45.9	45.2	45.0	45.0					

FIG. 5. TOTAL NITROGEN REMAINING IN THE KANSAS SOIL EACH YEAR AND THAT
RECOVERED IN THE CROPS

that is, at the end of each season there was less nitrogen in the soil and in the crop than was present in the soil at the end of the preceding year. At the end of the third year the greatest loss was apparent under the corn, wheat, oats, and corn, oats, wheat rotations. Under soybeans and cowpeas there was an increase in nitrogen the first year over that originally present. The second year there was a gain over that present in the soil at the end of the first year. The third year not as much nitrogen was recovered in the soil and crop as was present at the end of the second year.

FIG. 6. TOTAL NITROGEN REMAINING IN THE VIRGINIA SOIL EACH YEAR AND THAT RECOVERED IN THE CROPS

In the Virginia soil in all cases there was a progressive loss of nitrogen each year except with the legumes. Under the legumes the first year there was a definite gain. The second year there was more nitrogen recovered in the soil and crops than was in the soil at the end of the first year. The third year there was a loss with soybeans and a slight gain with cowpeas due probably to an increase in nitrogen found in the soil itself.

In tables 16, 17 and 18 are shown the amounts of nitrate nitrogen found in the soils after the removal of the crops and that in the fallows. In figures 7, 8 and 9 are shown both the nitrate nitrogen found in the soils after harvest and the nitrogen recovered in the crops, assuming that the nitrogen in the crops was removed from the soil as nitrate. Here, under all crops, in all soils except where legumes were grown, we find that there was a definite loss

TABLE 16

Nitrate nitrogen in soil after removal of crops

(California soil)

	1916			1917			1918	
Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Corn	0.39 0.34 0.37 0.28	0.34	Corn	0.25 0.17 0.22 0.19	0.21	Corn	0.17 0.25 0.12 0.17	0.18
Corn	0.26 0.27 0.25 0.28	0.26	Wheat	0.28 0.28 0.31 0.34	0.30	Oats	0.25 0.17 0.15 0.17	0.19
Corn	0.28 0.22 0.23 0.28	0.25	Oats	0.12 0.37 0.22 0.27	0.24	Wheat	0.25 0.25 0.25 0.25	0.25
Wheat	0.33 0.58 0.55 0.41	0.47	Wheat	0.37 0.39 0.30 0.31	0.34	Wheat	0.25 0.38 0.38 0.34	0.34
Oats	0.29 0.27 0.41 0.36	0.33	Oats	0.19 0.31 0.24 0.19	0.23	Oats	0.28 0.17 0.22 0.31	0.24
Cotton	0.13 0.12 0.14 0.09	0.12	Cotton	0.16 0.12 0.37 0.12	0.19	Cotton	0.25 0.17 0.17 0.17	0.19
Soybeans	0.50 0.51 0.62 0.39	0.50	Soybeans	0.22 0.19 0.27 0.19	0.22	Soybeans	0.22 0.31 0.25 0.25	0.26
Soybeans	0.40 0.44 0.47 0.40	0.43	Wheat	0.28 0.37 0.19 0.34	0.29	Oats	0.31 0.25 0.25 0.22	0.26
Soybeans	0.45 0.51 0.32 0.41	0.42	Oats	0.12 0.12 0.12 0.12	0.12	Wheat	0.17 0.17 0.30 0.17	0.20

TABLE 16—*Continued*

1916			1917			1918		
Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Cowpeas	0.84	0.82	Cowpeas	0.12	0.44	Cowpeas	0.12	0.22
	1.06			0.57			0.28	
	0.37			0.44			0.35	
	1.00			0.62			0.12	
Cowpeas	0.86	0.76	Soybeans	0.19	0.13	Cowpeas	0.28	0.21
	0.77			0.25			0.17	
	0.87			0.03			0.22	
	0.56			0.16			0.17	
Fallow check	1.12	0.98	Fallow check	1.50	1.42	Fallow check	2.06	1.96
	1.06			1.34			1.94	
	0.96			1.30			1.94	
	0.78			1.53			1.89	
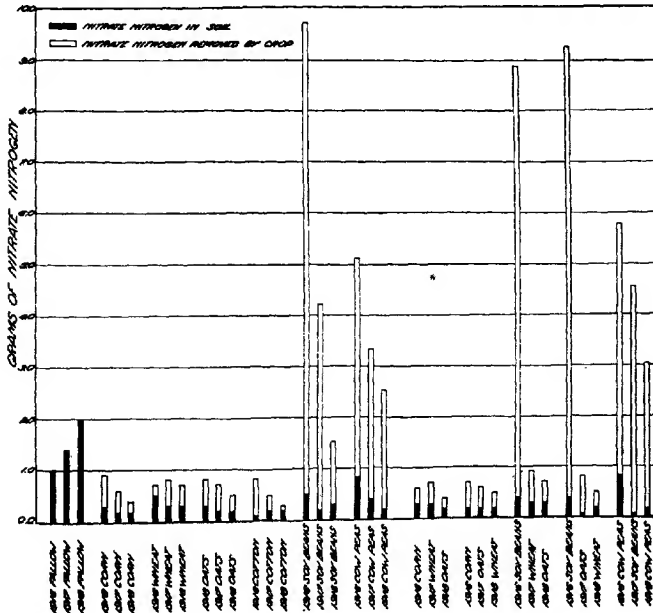
FIG. 7. NITRATE NITROGEN REMAINING IN THE CALIFORNIA SOIL EACH YEAR AND TOTAL NITROGEN RECOVERED IN THE CROPS

TABLE 17

Nitrate nitrogen in soil after removal of crops

(Kansas soil)

1916			1917			1918		
Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Corn	0.34 0.51 0.61 0.51	0.49	Corn	0.27 0.25 0.27 0.25	0.26	Corn	0.18 0.15 0.28 0.15	0.19
Corn	0.64 0.51 0.17 0.69	0.50	Wheat	0.54 0.64 0.25 0.63	0.51	Oats	0.47 0.74 0.35 0.74	0.57
Corn	0.67 0.56 0.56 0.67	0.61	Oats	0.31 0.34 0.17 0.05	0.22	Wheat	0.92 0.54 0.47 0.35	0.57
Wheat	1.26 1.22 0.91 1.36	1.19	Wheat	0.99 0.83 1.08	0.97	Wheat	1.14 0.84 0.84 1.24	1.01
Oats	0.62 0.69 0.59 1.00	0.72	Oats	0.19 0.17 0.20 0.26	0.20	Oats	0.56 0.54 0.39 0.39	0.47
Cotton	0.22 0.07 0.15 0.15	0.15	Cotton	0.37 0.32 0.42 0.42	0.38	Cotton	0.35 0.35 0.52 0.19	0.35
Soybeans	0.47 0.44 0.47 0.48	0.46	Soybeans	0.31 0.32 0.36 0.30	0.32	Soybeans	0.24 0.39 0.35 0.24	0.30
Soybeans	0.37 0.52 0.53 0.47	0.47	Wheat	0.71 0.77 0.96 0.63	0.77	Oats	0.84 0.79 0.79 0.56	0.74

TABLE 17—*Continued*

1916			1917			1918		
Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Soybeans	0.49	0.48	Oats	0.30	0.49	Wheat	1.18	0.99
	0.35			0.44			0.88	
	0.61			0.52			1.02	
	0.47			0.72			0.88	
Cowpeas	0.73	0.76	Cowpeas	0.43	0.53	Cowpeas	0.35	0.26
	0.80			0.54			0.28	
	0.61			0.48			0.14	
	0.89			0.67			0.27	
Cowpeas	0.47	0.54	Soybeans	0.24	0.15	Cowpeas	0.17	0.14
	0.41						0.17	
	0.68			0.05			0.14	
	0.62			0.15			0.07	
Fallow check	2.45	2.54	Fallow check	2.62	2.87	Fallow check	3.16	3.64
	2.76			2.82			3.60	
	2.45			3.25			3.95	
	2.49			2.80			3.87	

of nitrate nitrogen or, better, soluble nitrogen. These results bear out those reported by Lipman (2), Mooers (3), Russell (5), etc. Since no leaching was possible under the conditions of these experiments, this loss must be credited to volatilization of either free nitrogen or ammonia.

Returning to the subject of green manures, the results show an actual loss of nitrogen in all cases where non-legumes are grown and turned under as green manures. It is, of course, obvious that these results cannot be applied directly to field conditions. The inference seems warranted that the loss in nitrogen is due, to a considerable degree, to the thorough aeration given the different experimental soils; and, therefore, the losses of nitrogen that might be expected under field conditions presumably would be much less than indicated in the preceding experiments. Similarly, in field conditions where legumes are grown and turned under, while the general factors of relative gain and loss might be expected to obtain, the actual losses of nitrogen will probably be less.

CONCLUSIONS

Under the conditions of frequent handling with consequent thorough aeration of the experimental soils, the following results have been noted:

1. Cultivation or excessive aeration of a soil causes a loss of total nitrogen.

2. Under certain crops there is an absolute loss of nitrogen in excess of that recovered in the crops.
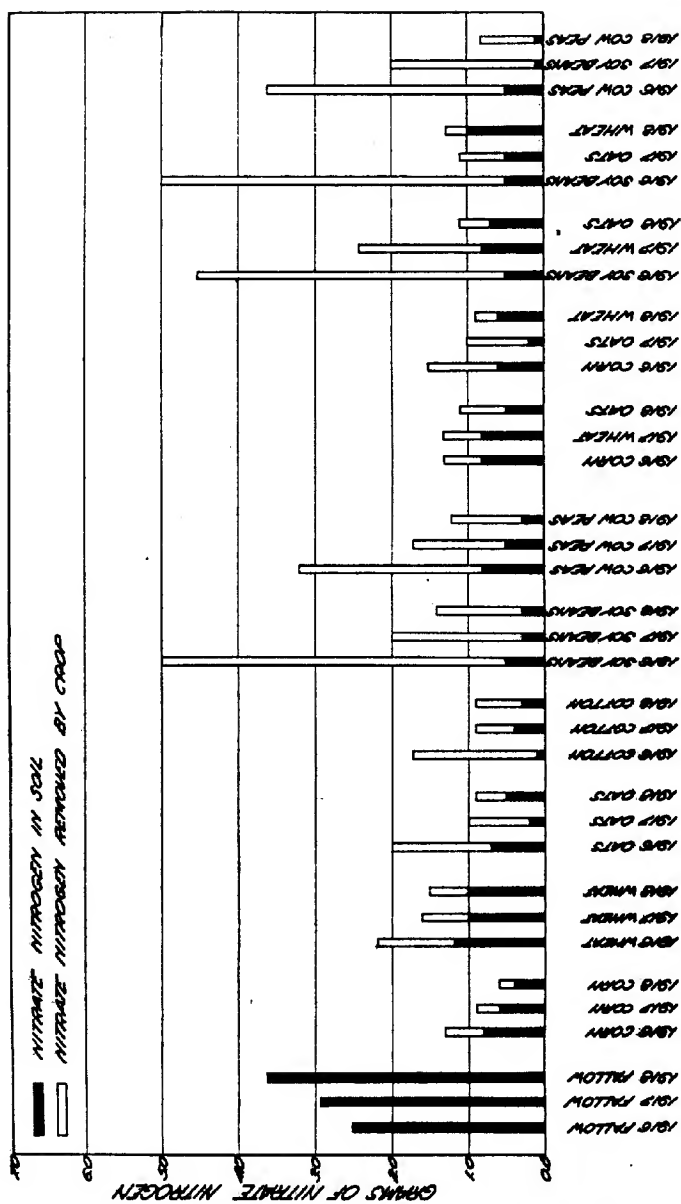
FIG. 8. NITRATE NITROGEN REMAINING IN THE KANSAS SOIL EACH YEAR AND TOTAL NITROGEN RECOVERED IN THE CROPS

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TABLE 18

Nitrate nitrogen in soil after removal of crops

(Virginia soil)

	1916			1917			1918	
Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*
Corn	1.69	1.36	Corn	0.18	0.14	Corn	0.25	0.17
	1.42			0.15			0.18	
	1.13			0.25			0.07	
	1.21			0.10			0.18	
Corn	1.50	1.33	Wheat	1.96	1.20	Oats	2.31	1.84
	1.31			0.55			1.20	
	1.21			1.00			1.80	
	1.31			1.31			2.06	
Corn	1.21	1.28	Oats	0.54	0.68	Wheat	1.64	1.78
	1.31			0.63			1.74	
	1.36			0.50			2.09	
	1.23			1.08			1.67	
Wheat	4.55	4.47	Wheat	3.85	4.81	Wheat	5.20	4.71
	5.10			6.90			5.30	
	4.59			4.86			4.35	
	3.63			3.65			4.00	
Oats	2.72	0.74	Oats	1.38	1.54	Oats	1.68	1.71
	2.47			1.36			0.98	
	3.10			1.80			2.31	
	2.66			1.63			1.86	
Cotton	0.77	0.66	Cotton	0.17	0.20	Cotton	0.57	0.66
	0.51			0.20			0.98	
	0.70			0.22			0.55	
	0.65			0.21			0.53	
Soybeans	0.97	0.86	Soybeans	0.29	0.26	Soybeans	0.24	0.21
	0.87			0.24			0.18	
	0.90			0.27			0.18	
	0.70			0.25			0.24	
Soybeans	1.29	1.00	Wheat	1.80	1.44	Oats	2.01	1.88
	1.01			0.99			1.76	
	0.95			2.02			2.09	
	0.75			0.94			1.66	
Soybeans	0.97	0.97	Oats	0.41	0.39	Wheat	1.58	1.51
	1.05			0.32			1.41	
	1.13			1.50			1.38	
	0.75			0.33			1.68	

TABLE 18—*Continued*

	1916			1917			1918	
Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average	Crop grown	Nitrate nitrogen remaining in soil	Average
	gm.	gm.		gm.	gm.		gm.	gm.
Cowpeas	0.55	0.68	Cowpeas	0.88	0.96	Cowpeas	2.01	1.84
	0.70			1.03			2.56	
	0.79			0.95			1.30	
	0.70			0.98			1.50	
Cowpeas	0.83	0.69	Soybeans	0.15	0.17	Cowpeas	0.25	0.42
				0.10			0.40	
	0.77			0.16			0.65	
	0.48			0.28			0.40	
Fallow Check	6.50	6.21	Fallow Check	9.20	8.77			
	6.30			10.00				
	5.90			7.50				
	6.16			8.40				
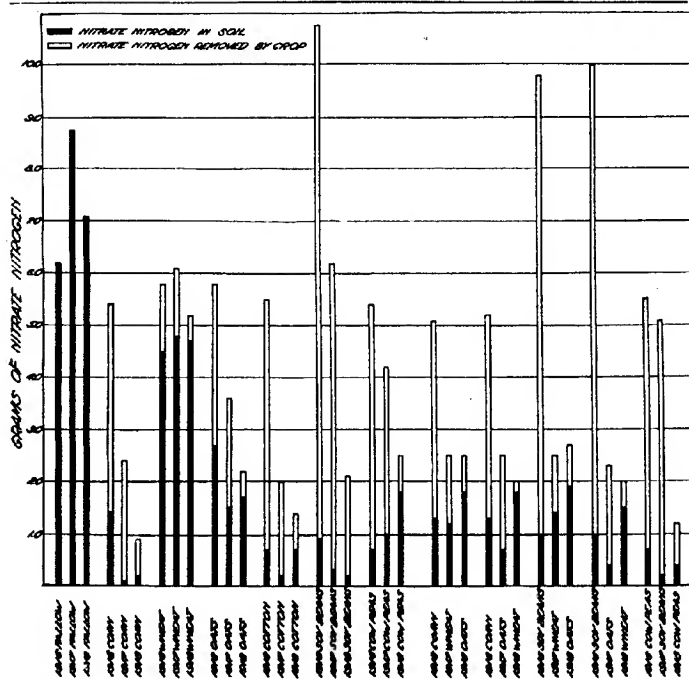
FIG. 9. NITRATE NITROGEN REMAINING IN THE VIRGINIA SOIL EACH YEAR AND TOTAL
NITROGEN RECOVERED IN THE CROPS

3. This varies with different crops and different soils.

4. This loss occurs under certain legumes as well as non-legumes.

5. When there is nitrogen fixation with the growth of certain legumes—that is, when there is recovered more than was taken from the soil—this nitrogen is found in the crop above the ground and if this is removed, the soil will have been depleted just as if a non-leguminous crop had been grown and removed.

6. It is recognized that these results are not directly applicable to field conditions. It is not improbable, however, that the changes found to occur under the experimental conditions indicate relatively similar, although perhaps much less marked, changes in the nitrogen content of the field soils when different crops are grown.

REFERENCES

(1) LIPMAN, C. B., AND SHARP, L. T. 1912 Toxic effects of "alkali salts" in soils on soil bacteria: III. Nitrogen fixation. *In* Centbl. Bakt. (etc.), Abt., 2, Bd. 35, p. 647–655.

(2) LIPMAN, J. G., AND BLAIR, A. W. 1916 Cylinder experiments relative to the utilization and accumulation of nitrogen. N. J. Agr. Exp. Sta. Bul. 289.

(3) MOOERS, C. A. 1917 A comparative study of the nitrogen economy of certain Tennessee soils. Tenn. Agr. Exp. Sta. Bul. 118.

(4) PIETERS, A. J. 1917 Green manuring: a review of the American experiment station literature. *In* Jour. Amer. Soc. Agron., v. 9, no. 2, p. 62–82; no. 3, p. 109–126; no. 4, p. 162–172.

(5) RUSSELL, E. J. 1912 Soil Conditions and Plant Growth. Longmans, Green and Company, London.

(6) SHUTT, F. T. Some characteristics of the Western Prairie soils of Canada. *In* Jour. Agr. Sci., v. 3, pt. 4, p. 335–357.

(7) SWANSON, C. O. 1917 The effect of prolonged growing of alfalfa on the nitrogen content of the soil. *In* Jour. Amer. Soc. Agron., v. 9, no. 7, p. 305–314.

(8) WRIGHT, R. C. 1915 The influence of certain organic materials upon the transformation of soil nitrogen. *In* Jour. Amer. Soc. Agron., v. 7, no. 5, p. 193–208.

(9) WRIGHT, R. C. 1916 Growing plants in large containers under control conditions. *In* Jour. Amer. Soc. Agron., v. 8, no. 2, p. 113–116.

CROSS-INOCULATION STUDIES WITH THE NODULE BACTERIA OF LIMA BEANS, NAVY BEANS, COWPEAS AND OTHERS OF THE COWPEA GROUP

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INTRODUCTION

In the spring of 1917, desiring to obtain a reliable source of inoculation for garden beans, three jars of sand, heavily inoculated with soil from a garden where navy and kidney beans were known to have been naturally inoculated, were planted to navy beans, kidney beans and lima beans, respectively. The roots of the navy and kidney beans thus planted were found to be abundantly supplied with nodules, but on the lima beans none were found. The nodules from the navy and kidney beans were crushed in water and returned to their respective jars, the latter then being seeded to lima beans. No nodules were produced although the plants reached a mature age. The third jar, which had contained uninoculated lima beans, was seeded to a mixture of kidney and navy beans, which on examination were found to have numerous nodules in every case.

The results indicated that the bacteria in kidney and navy bean nodules are different from those in lima beans. However, these tests were not made under controlled conditions, and it was desirable to repeat the experiment under sterile conditions, with pure cultures of the several nodule bacteria in question.

EXPERIMENTAL

Local garden soils did not yield a source of inoculation for lima beans, and a "pure culture" from a commercial company failed. Finally, soil which had grown inoculated lima beans was secured from California, nodules were produced by growing plants in sterile sand to which this soil was added and pure cultures obtained by plating out the nodules produced. One culture (1899) came from these original plates, made October, 1917, the other three (1896, 1897 and 1898) were obtained from nodules on a lima bean plant grown in the greenhouse, but the original source of inoculation is the same. These were isolated in February, 1919.

The pure cultures of navy bean bacteria used in the first experiment (1156 and 1157, table 1) were isolated in February, 1919, from a nodule on a navy

bean plant grown in the greenhouse. Because of the unexpected results of this experiment new isolations were made (1900, 1901, 1912 and 1913) in October, 1919, from a naturally inoculated navy bean plant dug from a local garden. The later isolations were used in the subsequent experiments as indicated, though behaving exactly as did the first two cultures.

Five cultures of cowpea bacteria were used, no. 1867 and 1868 were isolated in May, 1917, and no. 1153, 1869, and 1870 in January, 1919. These were tested out fully prior to these experiments. The plants from which they were obtained were grown in soil in the greenhouse.

In table 1 are reported the results of the first test with lima beans grown in beakers of sterile sand of 600 cc. capacity. In all the experiments sterilized seeds were employed. The methods used are the same as described by Burrill and Hansen (1). This experiment was started August 14 and ended September 7, 1919. Nodules were produced on lima beans by the lima-bean cultures

TABLE 1

Cross-inoculation of lima beans with navy-bean and cowpea bacteria

POT NUMBER	SEEDS PLANTED	SOURCE OF INOCULATION	NUMBER OF SEEDS	PLANTS OBTAINED	NODULE RECORD
1	Lima bean	Cowpea (1869)	4	4	No nodules
2	Lima bean	Cowpea (1153)	4	4	No nodules
3	Lima bean	Navy bean (1156)	4	4	No nodules
4	Lima bean	Navy bean (1157)	4	4	No nodules
5	Lima bean	Lima bean (1896)	4	4	Nodules very large and abundant
6	Lima bean	Lima bean (1898)	4	4	Nodules very large and abundant
7	Lima bean	Check, uninoculated	4	4	No nodules
8	Lima bean	Check, uninoculated	4	4	No nodules

only. Navy bean bacteria did not produce nodules on the lima bean, confirming our preliminary experiments. Cowpea bacteria were included because in a preliminary test lima-bean bacteria produced nodules on cowpeas. But the reciprocal cross failed, as indicated in the tabulated results. This should be carefully noted, as the explanation for it will become apparent in our further discussion.

Another experiment was conducted on a larger scale, four isolations each from cowpeas, lima beans and navy beans, being used. The seeds were planted on December 20, and the plants examined January 12, 1920. In this case the new isolations from a naturally infected navy bean plant were used. The results are shown in table 2, and confirmed the first test.

A similar experiment was tried with cowpea plants, the same cultures being used as before described. The seeds were planted November 22 and the plants examined December 23, 1919. The lima-bean bacteria produced nodules abundantly on cowpea roots, confirming the preliminary test. The cowpea

bacteria produced nodules on the host also, proving the viability of these bacteria. The navy bean cultures failed, however, proving that they were different from either cowpea or lima-bean bacteria. Again attention is directed to the failure of the cowpea cultures to produce nodules on the lima bean.

To demonstrate further the facts brought out, another experiment was started January 15, the results of which appear in table 4. The previous work was confirmed, in that the lima-bean bacteria produced nodules abundantly on cowpea roots, but again cowpea bacteria were unable to inoculate the lima beans as tested up to this time.

TABLE 2

Cross-inoculation of lima beans with navy-bean and cowpea bacteria

POT NUMBER	SEEDS PLANTED	SOURCE OF INOCULATION	NUMBER OF SEEDS	PLANTS OBTAINED	NODULE RECORD
1	Lima bean	Navy bean (1900)	4	4	No nodules
2	Lima bean	Navy bean (1901)	4	4	No nodules
3	Lima bean	Navy bean (1912)	4	4	No nodules
4	Lima bean	Navy bean (1913)	4	3	No nodules
5	Lima bean	Lima bean (1896)	4	4	Nodules very large and numerous
6	Lima bean	Lima bean (1897)	4	4	Nodules very large and numerous
7	Lima bean	Lima bean (1898)	4	3	Nodules very large and numerous
8	Lima bean	Lima bean (1899)	4	4	Nodules very large and numerous
9	Lima bean	Cowpea (1867)	4	4	No nodules
10	Lima bean	Cowpea (1868)	4	4	No nodules
11	Lima bean	Cowpea (1869)	4	4	No nodules
12	Lima bean	Cowpea (1870)	4	4	No nodules
13	Lima bean	Check, uninoculated	4	4	No nodules
14	Lima bean	Check, uninoculated	4	4	No nodules
15	Lima bean	Check, uninoculated	4	4	No nodules
16	Lima bean	Check, uninoculated	4	3	No nodules

Difficulty was experienced in trying to grow navy beans. Several attempts were made, but the germination of the seeds was poor, and those which germinated grew slowly. This was thought to be due to keeping the pots too wet. One-pint mason jars were used, and it was impossible to maintain proper moisture conditions. In order, therefore, to test out the navy bean cultures used, five 1-gallon battery jars of sterile sand were seeded to beans, five seeds being planted in each pot, one each of Early Red Valentine, Golden Wax, and Black Wax and two of common navy beans.

Three of the four cultures used successfully inoculated all the varieties in every case where the seeds germinated. The failure of culture 1900 to produce nodules on any of the five plants cannot be explained. This jar was badly

TABLE 3

Cross-inoculation of cowpeas with navy-bean and lima-bean bacteria

POT NUMBER	SEEDS PLANTED	SOURCE OF INOCULATION	NUMBER OF SEEDS	PLANTS OBTAINED	NODULE RECORD
1	Cowpea	Navy bean (1900)	4	3	No nodules
2	Cowpea	Navy bean (1901)	4	2	No nodules
3	Cowpea	Navy bean (1912)	4	4	No nodules
4	Cowpea	Navy bean (1913)	4	2	No nodules
5	Cowpea	Lima bean (1896)	4	4	Nodules very large and numerous
6	Cowpea	Lima bean (1897)	4	4	Nodules very large and numerous
7	Cowpea	Lima bean (1898)	4	4	Nodules very large and. numerous
8	Cowpea	Lima bean (1899)	4	4	Nodules very large and numerous
9	Cowpea	Cowpea (1868)	4	3	Nodules very large and numerous
10	Cowpea	Cowpea (1868)	4	4	Nodules very large and numerous
11	Cowpea	Cowpea (1869)	4	4	Nodules very large and numerous
12	Cowpea	Cowpea (1870)	4	3	Nodules very large and numerous
13	Cowpea	Check, uninoculated	4	4	No nodules
14	Cowpea	Check, uninoculated	4	3	Several scattered nodules
15	Cowpea	Check, uninoculated	4	3	No nodules
16	Cowpea	Check, uninoculated	4	3	No nodules

TABLE 4

Cowpea bacteria and lima-bean bacteria crossed on cowpeas and lima beans

POT NUMBER	SEEDS PLANTED	SOURCE OF INOCULATION	NUMBER OF SEEDS	PLANTS OBTAINED	NODULE RECORD
1	Cowpea	Lima bean (1896)	4	4	Nodules large and numerous
2	Cowpea	Lima bean (1897)	4	3	Nodules large and numerous
3	Cowpea	Lima bean (1898)	4	4	Nodules large and numerous
4	Cowpea	Lima bean (1899)	4	4	Nodules large and numerous
5	Lima bean	Cowpea (1867)	4	3	No nodules
6	Lima bean	Cowpea (1868)	4	3	No nodules
7	Lima bean	Cowpea (1869)	4	2	No nodules
8	Lima bean	Cowpea (1870)	4	3	No nodules

cracked in sterilization and it was put aside, but after the others had been prepared, it was again added to the series. It is possible that in the change of plans the bacteria were not added.

The results of this test are confirmatory of previous work in that varieties of common beans (not including lima beans) are inoculated by the same strain of nodule bacteria.

In table 6 is given the complete performance record of the navy-bean cultures used. The data reported in tables 1, 2, 3 and 5 are included together with the data from two other experiments not reported in detail. As before stated,

TABLE 5

Navy-bean cultures tested on bean varieties

POT NUMBER	PLANT	VARIETY OF BEAN	SOURCE OF INOCULATION	NODULE RECORD
1	a	Early Red Valentine	Navy bean (1900)	No nodules
	b	Golden Wax		No nodules
	c	Navy bean		No nodules
	d	Navy bean		No nodules
	e	Black Wax		No nodules
2	a	Early Red Valentine	Navy bean (1901)	Failed to germinate
	b	Golden Wax		Nodules large and numerous
	c	Navy bean		Nodules large and numerous
	d	Navy bean		Nodules large and numerous
	e	Black Wax		Nodules large and numerous
3	a	Early Red Valentine	Navy bean (1912)	Nodules large and numerous
	b	Golden Wax		Nodules large and numerous
	c	Navy bean		Nodules large and numerous
	d	Navy bean		Nodules large and numerous
	e	Black Wax		Failed to germinate
4	a	Early Red Valentine	Navy bean (1913)	Nodules small but numerous
	b	Golden Wax		Failed to germinate
	c	Navy bean		Nodules small but numerous
	d	Navy bean		Nodules small but numerous
	e	Black Wax		Failed to germinate

poor germination was the rule with navy beans and in several instances the plants which were obtained were poor and cannot be considered a fair test. As a whole, however, the results are trustworthy. With three of the cultures on the host plant, the results are positive. In no case herein reported did the navy-bean bacteria produce nodules on lima beans or cowpeas.

The data presented demonstrate that the bacteria causing the nodules on the roots of navy beans are different from those on lima beans. The varieties of common beans tested (Early Red Valentine, Golden Wax, Black Wax and common navy bean), however, are inoculated by navy-bean bacteria.

Apparently the limas stand apart. This is the first case known to the authors where different species within a given plant genus require different bacteria.

The data up to this point indicated that cowpea bacteria could not inoculate lima beans but that lima-bean bacteria easily produced nodules on the cowpea. This peculiar failure of the cowpea bacteria to cross on the lima bean could not be satisfactorily explained on any basis known and called for a repetition of the experiments. The purity of the cultures used was proved beyond doubt by all the methods known.

TABLE 6

Performance record of navy-bean cultures

CULTURE NUMBER	SEEDS PLANTED	NUMBER OF SEEDS	PLANTS OBTAINED	DATE EXAMINED	NODULE RECORD
1900	Navy bean	4	1	December 11, 1919	Nodules small but numerous
1900	Cowpea	4	3	December 23, 1919	No nodules
1900	Lima bean	4	4	January 12, 1920	No nodules
1900	Navy bean	4	2	January 12, 1920	No nodules, plant very poor
1900	Navy bean	5	5	February 14, 1920	No nodules
1901	Navy bean	4	2	December 11, 1919	Nodules large and numerous
1901	Cowpea	4	2	December 23, 1919	No nodules
1901	Lima bean	4	4	January 12, 1920	No nodules
1901	Navy bean	4	4	January 12, 1920	Nodules numerous
1901	Navy bean	5	4	February 14, 1920	Nodules large and numerous
1912	Navy bean	4	0		Failed to germinate
1912	Cowpea	4	4	December 23, 1919	No nodules
1912	Lima bean	4	4	January 12, 1920	No nodules
1912	Navy bean	4	1	January 12, 1920	No nodules, very poor plant
1912	Navy bean	5	4	February 14, 1920	Nodules large and numerous
1913	Navy bean	4	0		Failed to germinate
1913	Cowpea	4	2	December 23, 1919	No nodules
1913	Lima bean	4	3	January 12, 1920	No nodules
1913	Navy bean	4	0		Failed to germinate
1913	Navy bean	5	3	February 14, 1920	Nodules small but numerous

Because of our observations as to the relative appearance and growth of nodules on cowpeas and soybeans it was thought that the lima bean, like the soybean, might be slower than the cowpea in demonstrating nodule development. The rapid development of the nodules appears to be related to the nitrogen content of the seed and the rate of growth of the plant.

An experiment was outlined to test the cross-inoculation of cowpea cultures and other cultures of the cowpea group such as those of the partridge pea (*Cassia Chamaecrista*), peanut (*Arachis hypogoea*) and Japan clover (*Lespedeza striata*) on the lima bean.

The data obtained are included in table 7. The plants made a good growth in this experiment. All the cultures produced nodules on the lima bean except *Cassia* and cowpea 1911. The results of this experiment demonstrated that the bacteria of certain plants in the cowpea group could produce nodules on lima beans. The failure in this experiment of the *Cassia* and cowpea culture 1911 to produce nodules on lima beans was in agreement with the performance of certain cowpea cultures in the earlier experiments. It was thought that there might be a difference in the rate of infection between different cultures

TABLE 7

Bacteria of cowpea group crossed on lima bean

JAR NUMBER	SEEDS PLANTED	SOURCE OF INOCULATION	DATE PLANTED	DATE HARVESTED	NUMBER OF SEEDS	PLANTS OBTAINED	NODULE RECORD
34	Cowpea	Cowpea (1456)	March 24, 1920	April 21, 1920	5	1	Large nodules
35	Cowpea	Cowpea (1459)	March 24, 1920	April 21, 1920	5	2	Numerous nodules
36	Lima bean	Partridge pea (1460)	March 24, 1920	April 21, 1920	5	3	No nodules
37	Lima bean	Partridge pea (1461)	March 24, 1920	April 21, 1920	5	3	No nodules
38	Lima bean	Peanut (1462)	March 24, 1920	April 21, 1920	5	3	Large numerous nodules
39	Lima bean	Peanut (1462)	March 24, 1920	April 21, 1920	5	3	Large numerous nodules
40	Lima bean	Peanut (1464)	March 24, 1920	April 21, 1920	5	2	Large numerous nodules
41	Lima bean	Peanut (1465)	March 24, 1920	April 21, 1920	5	2	Large numerous nodules
42	Lima bean	Japan clover (1449)	March 24, 1920	April 21, 1920	5	4	Large numerous nodules
43	Lima bean	Japan clover (1450)	March 24, 1920	April 21, 1920	5	3	Large numerous nodules
44	Lima bean	Cowpea (1911)	March 24, 1920	April 21, 1920	5	3	No nodules
45	Lima bean	Cowpea (1457)	March 24, 1920	April 21, 1920	5	3	Large nodules
46	Lima bean	Check, uninoculated	March 24, 1920	April 21, 1920	5	4	No nodules
47	Lima bean	Check, uninoculated	March 24, 1920	April 21, 1920	5	3	No nodules
48	Cowpea	Check, uninoculated	March 24, 1920	April 21, 1920	5	4	No nodules
49	Cowpea	Check, uninoculated	March 24, 1920	April 21, 1920	5	3	No nodules

or that some condition of these particular jars might be the cause of no nodule production.

In order still further to verify the results obtained, an experiment was conducted that included cowpea, partridge-pea, Japan-clover, peanut and beggar-weed (*Desmodium tortuosium*) cultures of various transfers. *Radiobacter* isolated from cowpea nodules were introduced to test its purity. Some of these were used in the preceding experiments while others were introduced here for the first time.

TABLE 8

Bacteria of cowpea group crossed on lima beans

JAR NUMBER	SEEDS PLANTED	SOURCE OF INOCULATION	DATE PLANTED	DATE HARVESTED	NUMBER OF SEEDS	PLANTS OBTAINED	NODULE RECORD
82	Cowpea	Check, uninoculated	May 1, 1920	May 25, 1920	5	4	No nodules
83	Cowpea	Check, uninoculated	May 1, 1920	May 25, 1920	5	5	No nodules
84	Lima bean	Check, uninoculated	May 1, 1920	May 25, 1920	5	5	No nodules
85	Lima bean	Check, uninoculated	May 1, 1920	May 25, 1920	5	5	No nodules
86	Lima bean	Cowpea (1557)	May 1, 1920	May 25, 1920	5	5	Numerous nodules
87	Lima bean	Cowpea (1557)	May 1, 1920	May 25, 1920	5	5	Numerous nodules
98	Lima bean	Cowpea (1556)	May 1, 1920	May 25, 1920	5	4	Large numerous nodules
99	Lima bean	Cowpea (1558)	May 1, 1920	May 25, 1920	5	4	Large numerous nodules
100	Lima bean	Cowpea (1559)	May 1, 1920	May 25, 1920	5	5	Large numerous nodules
101	Lima bean	Cowpea (1911)	May 1, 1920	May 25, 1920	5	4	Large numerous nodules
90	Lima bean	Partridge pea (1551)	May 1, 1920	May 25, 1920	5	4	Small numerous nodules
91	Lima bean	Partridge pea (1551)	May 1, 1920	May 25, 1920	5	5	Small numerous nodules.
102	Lima bean	Partridge pea (1460)	May 1, 1920	May 25, 1920	5	5	Numerous nodules
103	Lima bean	Partridge pea (1461)	May 1, 1920	May 25, 1920	5	4	Numerous nodules
92	Lima bean	Japan clover (1552)	May 1, 1920	May 25, 1920	5	4	Large nodules
93	Lima bean	Japan clover (1552)	May 1, 1920	May 17, 1920	5	4	Small nodules just started
108	Lima bean	Japan clover (1552)	May 1, 1920	May 25, 1920	5	5	Large numerous nodules
109	Lima bean	Japan clover (1553)	May 1, 1920	May 25, 1920	5	5	Large numerous nodules
94	Lima bean	Peanut (1562)	May 1, 1920	May 25, 1920	5	4	Large numerous nodules
95	Lima bean	Peanut (1562)	May 1, 1920	May 25, 1920	5	5	Large numerous nodules
104	Lima bean	Peanut (1564)	May 1, 1920	May 25, 1920	5	3	Large numerous nodules
105	Lima bean	Peanut (1565)	May 1, 1920	May 25, 1920	5	5	Large numerous nodules
96	Lima bean	Beggar-weed (1555)	May 1, 1920	May 25, 1920	5	4	Large numerous nodules
97	Lima bean	Beggar-weed (1555)	May 1, 1920	May 25, 1920	5	4	Large numerous nodules
106	Lima bean	Beggar-weed (1452)	May 1, 1920	May 25, 1920	5	5	Large numerous nodules
107	Lima bean	Beggar-weed (1452)	May 1, 1920	May 25, 1920	5	5	Large numerous nodules
88	Lima bean	Radiobacter (7)	May 1, 1920	May 25, 1920	5	4	No nodules
89	Lima bean	Radiobacter (7)	May 1, 1920	May 25, 1920	5	4	No nodules
110	Lima bean	Radiobacter (1974)	May 1, 1920	May 25, 1920	5	5	No nodules
111	Lima bean	Radiobacter (1974)	May 1, 1920	May 25, 1920	5	5	No nodules

The complete data are found in table 8. In this experiment, which was conducted under ideal growing conditions, all the cultures produced nodules on the lima bean, except *radiobacter*. The nodules produced by *Cassia* were smaller than those of the other cultures. This experiment confirms the previous one and proves that the bacteria of the members of the cowpea group tested cross on the lima beans.

It was observed that nodule production on the lima bean was slower than that observed on many other plants studied. This observation offered a possible explanation of the negative results obtained with certain of the cowpea and *Cassia* cultures. It will be noted that cultures or transfers which earlier failed to produce nodules, all caused excellent nodule production in these later experiments.

An opportunity to test the relative size of nodules produced under identical conditions of growth on cowpeas and lima beans was presented in connection with other experiments. Lima beans and cowpeas were planted in the same jars of sterile sand. In some treatments bacteria from cowpea cultures and in other treatments bacteria from lima-bean cultures were applied as the inoculation for both kinds of plants. Nodules were larger on the cowpeas than on the lima beans of the same age. The general tendency for larger nodules on the cowpea than on the lima bean, at the earlier stages of growth, is in agreement with similar observations made between the nodule sizes of the cowpea and the soybean. The explanation offered for the difference in nodule size is based upon the relative nitrogen contents of the cowpea and lima bean seeds. The nitrogen content of lima-bean seeds is approximately twice that of the cowpea seeds. This fact coupled with the slower rate of growth of the lima bean would delay the need for atmospheric nitrogen and consequently not hasten the nodule development.

DISCUSSION

These experiments further emphasize the profitable field of investigation that is still open in the study of legume bacteria from the standpoint of cross-inoculation. No fundamental relationship has yet been approached on which any satisfactory explanation for the grouping of legume bacteria for inoculation purposes can be based.

To repeat an earlier statement this work reports the first definite case known to the authors where all the species within a given plant genus are not inoculated by the same nodule bacteria. No reason can be given at this time for the apparent exception, although it is recognized that the metabolism of the lima bean is unlike that of the other beans studied. That other cases of this kind exist seems probable and no doubt further study will discover such.

It had previously been shown (1, p. 135, 136) that cowpeas are inoculated by nodule bacteria from 17 plant species, representing 9 plant genera, several of which (*Cassia*, *Acacia* and *Vigna*) stand widely apart botanically. It has been shown now that the nodule bacteria of another species (*Phaseolus lunatus*, lima bean) of another genus cross-inoculates with the cowpea.

CONCLUSIONS

1. The nodule bacteria of the lima bean (*Phaseolus lunatus*) are distinct from those of the navy and kidney beans (*Phaseolus vulgaris*) for inoculation purposes.

2. The nodule bacteria of lima beans are identical with those of the cowpea group for inoculation purposes.

REFERENCE

(1) BURRILL, T. J., AND HANSEN, R. 1919 Is symbiosis possible between legume bacteria and non-legume plants? Ill. Agr. Exp. Sta. Bul. 202.

THE INFLUENCE OF SOIL REACTION ON THE GROWTH
OF ALFALFA[1]

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The application of hydrogen-ion concentration measurements in problems of soil fertility and plant growth is of only recent origin. The importance of such studies has been pointed out by Gillespie (2) who was the first to make use of the hydrogen electrode on a somewhat extensive scale to measure the hydrogen-ion concentrations of soils in suspension. Later Gillespie and Hurst (4) and Gillespie (3) measured the hydrogen-ion concentrations of soil extracts or suspensions in water by a simple colorimetric method, stating that the two methods agreed sufficiently well to show that either one yields approximately correct results. Sharp and Hoagland (12) also discuss the importance of hydrogen-ion concentration measurements in connection with soil fertility problems. Plummer (11), Wherry (13), Knight (8), Joffe (7), and others have made hydrogen-ion concentration measurements of soil suspensions or water extracts of soils either by the use of the hydrogen electrode or by the simpler colorimetric method in connection with soil fertility investigations or in studying other soil phenomena.

Studies relating to the specific effects of the hydrogen-ion concentration on the growth of agricultural plants are indeed limited. Hoagland (6) studied the effect of hydrogen-ion concentration in nutrient solutions on the growth of barley seedlings, and points out that comparatively few experiments have been recorded which bear directly on the growth of the plants of agricultural importance as affected by the acidity or alkalinity of the media in which they were grown.

An attempt has here been made to adjust the soil reaction, as indicated by hydrogen-ion determinations, of a single soil type by the use of sulfuric acid and calcium carbonate and to study experimentally in a very limited way the influence of specific soil reactions upon the growth of alfalfa plants. Alfalfa was chosen for this work because of its economic importance and because of its supposed sensitiveness to soil acidity.

The work was carried out at the laboratory of plant physiology of the New Jersey Agricultural Experiment Station. It is a pleasure here to express thanks to Dr. J. W. Shive for his supervision and interest in the work, also to Dr. S. Waksman for valuable suggestions.

EXPERIMENTAL PROCEDURE

The soil used in these tests was of the Penn loam type obtained from one of the experimental plots of the New Jersey Agricultural Experiment Station, on which alfalfa was grown. The water extract of this soil gave a hydrogen-ion exponent (pH value) of 6.4. The first step in the preparation of the soil cultures was to establish a range of soil reactions both above and below that of the initial hydrogen-ion exponent value of the soil used. To accomplish this, preliminary tests were made with soil in drinking glasses. Fifty grams of the air-dry soil were placed in each glass. To change the reaction of the soil so as to obtain a series of cultures having a range in hydrogen-ion exponent values below the original value of the soil used (pH 6.4), sulfuric acid (C. P., specific gravity 1.84) in varying amounts was thoroughly mixed with the soil in the glasses after diluting with a sufficient amount of distilled water to give to the soil a moisture content approximately optimum for plant growth. In this way a series of ten cultures was obtained varying in hydrogen-ion exponent values of the soil extracts from 6.4 to 3.0. In another series of ten cultures, precipitated calcium carbonate (99 per cent pure) in varying amounts was added to the soil with thorough mixing in order to obtain a range in the hydrogen-ion exponent values of the soil extracts above the original value. By this method the reaction of the soil in the glasses was changed to give hydrogen-ion exponent values varying from 6.4 to 7.1.

The soil in the glasses was kept at approximately the optimum moisture content for plant growth, and daily determinations were made of the hydrogen-ion concentration of water extracts of the soil in the various glasses. After a period of from 7 to 14 days, the reaction of the treated soil, as indicated by the hydrogen-ion exponents, became constant and no further changes were observed. The water extracts were prepared according to the method recommended and used by Gillespie and Hurst (4). The hydrogen-ion concentrations of the soil extracts were determined by the colorimetric method following the work of Clark and Lubs (1) in the preparation of the standard buffer mixtures and in the use of indicators.

Glazed earthenware pots each having a capacity of 1 gallon were used as containers. Seven pounds of air-dry soil taken from the same lot as that which was used in the preliminary tests with drinking glasses was placed in each container.

The amounts of sulfuric acid or of calcium carbonate to be added to the soil in the containers in order to obtain a series of soil cultures giving the desired range in the hydrogen-ion exponent values, were calculated from the data of the preliminary experiments. The amounts of sulfuric acid added to the soil in the various pots ranged from 15.5 cc. to 0.44 cc. This was always diluted with the distilled water which was applied to the soil to give the desired initial moisture content which was the same in all the pots. The calcium carbonate was thoroughly mixed with the air-dry soil and the distilled water was added

afterward.   The amounts of calcium carbonate added to the soil in the various pots ranged from 1.0 to 8.05 gm. per pot.   A series of 18 treated cultures and 2 checks, untreated, was thus prepared, giving a range in hydrogen-ion exponent values varying at somewhat irregular intervals from 3.0 to 7.1.   The pots were allowed to stand in the greenhouse unplanted until the hydrogen-ion exponent values of the soil extracts remained approximately constant, as indicated by the tests made from time to time.

The soil in each of the pots was planted with 50 seeds and after germination some of the young plants were removed, leaving 10 plants in each pot.   The cultures were weighed every other day and at each weighing the water lost by evaporation from the surface of the soil and by transpiration was restored by the addition of distilled water.   The cultures were conducted from October 28, 1919 to May 5, 1920.   Hydrogen-ion concentration measurements of the soil from each culture were made at regular intervals throughout the growth period; in all 12 determinations for each culture in the series were made.   At the end of the growth period the plants were harvested, and the dry weights of tops, per cent of nitrogen in the tops, and the relative number of nodules on the roots of the plants from the various cultures were determined.

EXPERIMENTAL RESULTS

The numerical data showing the relation between the soil reaction as indicated by the hydrogen-ion exponent values of the water extracts of the soil samples taken at regular intervals during the growth period, and the various quantitative plant measurements are presented in table 1.   In the table are given four columns of pH values, three of which represent the results of tests made at the beginning, near the middle, and at the end of the growth period, while the fourth column gives the averages of all the determinations (12 in number) for each culture.   These columns of pH values serve to show the general trend of the change in the reaction of the soil in the various cultures during the growth of the plants.

A comparison of the average pH values with the corresponding percentages of germination brings out the fact that the germination of the alfalfa seed was greatly reduced in the cultures showing average pH values of the soil extracts below 4.5.   In culture 1, the soil extract of which gave a pH value of 3.3, no germination occurred.   As the hydrogen-ion concentration of the soil decreased from a pH value of 3.5 to one of 4.5, a gradual increase in the percentage of germination is shown.   However, germination was remarkably constant in cultures showing a range in the average hydrogen-ion exponent values from 4.5 to 7.0.

The data of table 1 show very clearly that alfalfa may grow fairly well in a very acid soil, as is indicated by the growth of the plants in culture 4.   The soil in this culture gave an average hydrogen-ion exponent value of 3.8.   While only three plants survived this high hydrogen-ion concentration, the fact

remains that these three plants, after becoming established, made very good growth, and this is also true of the four surviving plants in culture 5, and the five surviving plants in culture 6. It is to be emphasized, however, that in these cultures and also in those with soils giving somewhat higher hydrogen-ion exponent values, the plants experienced difficulty in establishing themselves. During the first two months the plants were retarded in growth, making slow

TABLE 1

Numerical data showing the relation of soil reaction to germination, nodule formation, dry-weight yields of tops, and nitrogen content of alfalfa tops grown from October 29 to May 5 in soil cultures

CULTURE NUMBER	pH VALUES OF WATER EXTRACTS OF SOIL				GERMINA-TION	NODULES PER PLANT RELATIVE TO THOSE OF CULTURE 4 AS UNITY	DRY WEIGHT OF TOPS (10 PLANTS)	TOTAL NITROGEN IN TOPS
	October 28, original	January 28	May 5, final	Average of all (12) tests				
					per cent			*per cent*
1	3.0	3.5	3.5	3.3	0			
2	3.2	3.6	3.6	3.5	5			
3	3.4	3.8	3.8	3.6	28			
4*	3.6	3.8	4.2	3.8	60	1	3.7	1.85
5*	4.0	3.9	4.6	4.0	85	4	4.9	2.44
6*	4.1	4.2	4.8	4.3	90	2	6.2	2.94
7	4.4	4.4	5.0	4.5	94	1	6.7	2.89
8	4.8	5.0	6.0	5.0	94	3	6.2	3.08
9	5.6	5.8	6.0	5.7	94	3	6.4	3.03
Check	6.4	6.2	6.4	6.3	92	4	6.8	3.21
11	6.4	6.6	6.2	6.3	94	5	6.7	3.58
12	6.4	6.6	6.4	6.4	95	5	6.6	3.53
13	6.6	6.6	6.6	6.5	94	5	8.4	3.28
14	7.0	6.6	6.6	6.7	94	6	8.0	3.41
15	7.0	6.8	6.6	6.7	94	6	7.2	3.42
16	7.0	6.8	6.7	6.8	94	7	7.7	3.41
17	7.0	6.9	6.8	6.8	94	7	7.7	3.52
18	7.0	6.9	6.9	6.9	94	9	7.8	3.56
19	7.1	7.0	7.0	7.0	94	7	8.7	3.60
Check	6.4	6.4	6.4	6.3	94	5	6.8	3.14

* Three plants only survived in culture 4, four plants in culture 5, and five plants in culture 6. After becoming established the plants in these cultures made good growth.

progress. During the latter half of the growth period, however, these plants appeared to grow more rapidly than did those in the cultures the soil extracts of which had lower hydrogen-ion concentrations.

To bring out more clearly the relation of the hydrogen-ion concentrations of the soil extracts to the dry weights of alfalfa tops and to their nitrogen content, the values representing these measurements taken from the proper columns of table 1, were plotted to form the graphs of figure 1, the broad,

narrow, and dotted lines representing, respectively, the dry weights of tops, average pH values, and per cent of nitrogen in the alfalfa tops. The single set of ordinates may here refer to dry weights, pH values, or percentages of nitrogen when applied to the graphs representing these measurements.

The graph representing dry weights, although it is somewhat irregular, shows a general agreement with the graph of pH values, in sloping gradually upward to the right. This indicates a gradual increase in the yields of alfalfa tops with corresponding decrease in the hydrogen-ion concentration of the soil extracts from the various cultures. It will be observed, however, that cultures 13 and 14, for which average hydrogen-ion exponents of 6.5 and 6.7 are indicated, produced almost as high yields as did culture 19, showing an
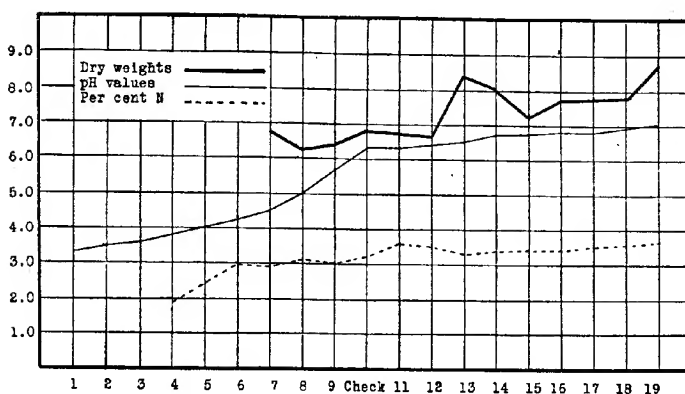
FIG. 1. GRAPHS SHOWING THE RELATION OF YIELDS AND PER CENT NITROGEN CONTENT OF ALFALFA TOPS TO THE REACTION OF THE SOIL IN WHICH THE PLANTS WERE GROWN

Ordinates represent grams, pH values, or percentages when referred to yields, soil reaction, or nitrogen content, respectively.

average hydrogen-ion exponent of 7.0. It is important here to note that the plants in the cultures with higher hydrogen-ion concentrations of the soil water, particularly in the cultures in which the soil reaction was adjusted by the use of sulfuric acid, showed better root development than did the plants in the cultures in which the soil water had a very low hydrogen-ion concentration or a neutral reaction. This is perhaps to be ascribed to the effect of the sulfates formed by the introduction of sulfuric acid into the soil. Hart and Tottingham (5) have shown that sulfates have an especially beneficial influence on root development, particularly on the roots of red clover, to which alfalfa bears a close relationship and may respond to the influence of sulfates in a similar manner.

A very interesting feature of the effect of soil reaction on the growth of alfalfa is its influence upon the nitrogen content of the plants. As the graphs of figure 1 clearly show, the nitrogen content of the plants increases correspondingly as the average hydrogen-ion exponents of the soil extracts from the different cultures increase. In a general way the same relation exists between the relative number of nodules formed on the roots of the plants in the various cultures and the hydrogen-ion exponents of the soil extracts, as may be observed from an examination of the data in table 1. Lipman and Blair (10) have pointed out that the nitrogen content of leguminous plants grown on unlimed plots is lower than is that of plants of the same species grown on limed plots. They correlate this phenomenon directly with the greater abundance of nodules on the roots of the plants grown on the limed plots, which indicates a greater capacity of the nodule-forming organisms for the fixation of atmospheric nitrogen, thus furnishing the plants with a greater supply of available nitrogen. This, however, does not explain the increased nitrogen content of non-leguminous plants grown on limed plots as compared with those grown on unlimed plots, a fact which was reported by these same authors in an earlier publication (9).

In this connection it might be suggested that the soil conditions which influence the activity of the nodule-forming organisms may in a like manner influence the nitrogen assimilation of non-leguminous as well as of leguminous plants. Whatever may be the true explanation, it appears that the reaction (hydrogen-ion concentration) of the medium in which the plants are grown has a direct influence upon the nitrogen content of the plants.

It is to be emphasized that no attempt was here made to determine the specific soil reaction as indicated by the hydrogen-ion exponents of the soil extracts to which the alfalfa plant responds best. To do this would necessitate extending the range of soil reactions to include hydrogen-ion exponent values considerably beyond the neutral point on the alkaline side. With the soil type here used this could not be accomplished without too greatly altering the proportions of the mineral constituents of the soil solution. It is, of course, quite probable that each soil type possesses a specific optimum or an optimum range of hydrogen-ion concentration values for the growth of alfalfa which is different from that of other soil types.

SUMMARY

Alfalfa was grown in pots containing soil the reaction of which was adjusted by the use of sulfuric acid and calcium carbonate. A series of 20 cultures was prepared showing a range in the hydrogen-ion concentrations of the soil extracts varying at somewhat irregular intervals from a pH value of 3.0 to one of 7.1. Water extracts of the soil from each culture were prepared at regular intervals during the growth of the plants and the hydrogen-ion concentrations determined by the colorimetric method.

1. The germination of alfalfa seeds was practically the same with pH values of the soil varying from 4.5 to 7.0, but was greatly reduced in cultures which yielded soil extracts having pH values below 4.5.

2. Yields of alfalfa tops showed a gradual increase with an increase in the pH values of the soil extracts from 3.8 to 7.0. The alfalfa plants experienced difficulty in becoming established in cultures yielding soil extracts with high hydrogen-ion concentrations, but after becoming established they showed normal green color, high vigor, and made excellent growth in soil having a pH value as low as 3.8.

3. With increasing hydrogen-ion concentration of the soil extracts, nodule formation on the roots of the plants was correspondingly less abundant.

4. Plants in the cultures which yielded soil extracts with very low hydrogen-ion concentrations showed poorer root development than did the plants in the cultures with higher hydrogen-ion concentrations of the soil extracts.

5. The nitrogen content of the plants showed a gradual increase with a corresponding decrease in the hydrogen-ion concentration of the soil extracts.

REFERENCES

(1) CLARK, W. M., AND LUBS, H. A. 1917 The colorimetric determination of hydrogen-ion concentration and its application in bacteriology. *In* Jour. Bact., v. 2, no. 1, p. 1–34; no. 2, p. 109–136; no. 3, p. 193–236.

(2) GILLESPIE, L. J. 1916 The reaction of soil and measurements of hydrogen-ion concentration. *In* Jour. Wash. Acad. Sci., v. 6, no. 1, p. 7–16.

(3) GILLESPIE, L. J. 1920 Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with special reference to soils. *In* Soil Sci., v. 9, no. 2, p. 115–136.

(4) GILLESPIE, L. J., AND HURST, L. A. 1917 Hydrogen-ion concentration measurements of soils of two types; Caribou loam and Washburn loam. *In* Soil Sci., v. 4, no. 4, p. 313–319.

(5) HART, E. B., AND TOTTINGHAM, W. E. 1915 Relation of sulfur compounds to plant nutrition. *In* Jour. Agr. Res., v. 5, no. 6, p. 233–250.

(6) HOAGLAND, D. R. 1917 The effect of hydrogen and hydroxyl-ion concentration on the growth of barley seedlings. *In* Soil Sci., v. 3, no. 6, p. 547–560.

(7) JOFFE, J. S. 1920 Hydrogen-ion concentration measurements of soils in connection with their "lime-requirements." *In* Soil Sci., v. 9, no. 4, p. 261–266.

(8) KNIGHT, H. G. 1920 Acidity and acidimetry of soils. 2. Investigation of acid soils by means of the hydrogen electrode. *In* Jour. Indus. Engin. Chem., v. 12, no. 5, p. 457–464.

(9) LIPMAN, J. G., AND BLAIR, A. W. 1915 Lime as a factor in the utilization of nitrogen. *In* N. J. Agr. Exp. Sta. 36th Ann. Rpt., p. 204.

(10) LIPMAN, J. G., AND BLAIR, A. W. 1918 The influence of lime upon the yield of dry matter and nitrogen from different varieties of soybeans. *In* N. J. Agr. Exp. Sta. 39th Ann. Rpt., p. 190.

(11) PLUMMER, J. K. 1918 Studies in soil reaction as indicated by the hydrogen electrode. *In* Jour. Agr. Res., v. 12, no. 1, p. 19–31.

(12) SHARP, L. T., AND HOAGLAND, D. R. 1916 Acidity and adsorption in soils as measured by the hydrogen electrode. *In* Jour. Agr. Res., v. 7, no. 3, p. 123–145.

(13) WHERRY, E. T. 1916 A chemical study of the habitat of the walking fern, *Camptosorus rhizophyllus* (L) Link. *In* Jour. Wash. Acad. Sci., v. 6, no. 20, p. 672–679.

THE EFFECT OF FERTILIZERS ON BLUEBERRIES[1]

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In a recent paper (1) the writer has shown the effect of certain fertilizers upon the yield of cranberries, in the course of experimental work conducted at Browns Mills, N. J., in 1919. A large blueberry plantation situated on the same property as the cranberry substation gave the writer an exceptional opportunity to observe the effect of certain commercial fertilizers applied to the blueberry. The blueberry (*Vaccinium corymbosum*) is a close relative of the cranberry (*Vacinium oxycoccus*). The commercial cultivation of the former is practically new, although the latter has been grown under artificial conditions for a half-century. The soil on which both plants are grown has a slightly acid reaction, and is usually more or less sandy and well irrigated.

Many papers have been published on the commercial culture of the blueberry by F. V. Coville, of the Bureau of Plant Industry, United States Department of Agriculture (2, 3, 4, 5,).

The blueberry plantation at Browns Mills is 4 to 5 years old. Early in the spring of 1919 the plants started slowly with a starved yellow appearance, and the large set of fruit buds showed no probability of maturing fruit unaided. An application of plant-food suggested itself as a means of increasing the active leaf surface of the plant, thereby increasing the fruiting possibilities. The writer was asked to recommend a mixture which, in his opinion, would be the most economical. Because of lack of space for experiments, it was necessary to reduce the problem to a choice among three substances. Experience with the cranberry plant led the writer to believe that the question was whether a quick acting mineral fertilizer or a more slowly available mixture was best suited to the needs of the blueberry plant, and also how much the plant was in need of nitrogen. Basing the work on this judgment the writer planned an experiment of five plots, arranged and treated as in table 1.

The fertilizer applied on these plots was purposely put on in large amounts in order to get results which could be interpreted within the shortest possible time. At the end of 2 weeks a very great difference was noted in the plant growth. The proportion of leaf surface to fruit had increased in all the plots receiving plant-food. In plot 2 the leaves and new stems showed

[1] Paper No. 7 of the Technical Series, New Jersey Agricultural Experiment Stations, Cranberry Substation.

the dark green color peculiar to plots having an overdose of nitrogen. Plots 3 and 5 both showed more foliage than plot 4 and the general appearance of plot 5 seemed to be best. From these early indications the writer recommended an application of 600 pounds of a mixture similar to that on no. 5. Throughout the year plots 3 and 5 had an exceptionally good appearance, growing larger and having larger berries than plot 4.

TABLE 1

Fertilizer treatments per acre on blueberries

PLOT NUMBER	TREATMENT
1	Nothing
2	250 lbs. nitrate of soda
3	250 lbs. nitrate of soda 750 lbs. acid phosphate 250 lbs. Nebraska potash
4	Nothing
5	170 lbs. nitrate of soda (15.20 per cent N) 230 lbs. dried blood (13.2 per cent N) 340 lbs. steamed bone (2.50 per cent N; 22.90 per cent P_2O_5) 340 lbs. phosphate rock (26.90 per cent P_2O_5) 170 lbs. Nebraska potash (28.5 per cent K_2O)

The yield in 1919 was not recorded because of lack of help at the bearing season. However, it was noted that individual berries on the plots receiving the fertilizer were much larger than on the check plots.

In the spring of 1920 the blossoms on plots 2 and 3 were so numerous that it was evident again that the bushes could not mature all the fruit. This was especially evident on plot 3. The bushes in plot 5, while they seemed to have a preponderance of blossoms over leaves, were in much better condition than those on plot 3.

The applications made in 1919 were repeated in 1920, except that plot 5 was divided into two parts and only half of the plot received the treatment. This made 6 plots. The crop yields were taken on three rows, each a different strain of blueberry, and the yields of plots 5 and 6 were doubled in order to make their yield comparable to the yields of the other plots of twice their size.

The detailed crop record is given in table 2.

The plots show that the fertilizer treatment did not hurry the ripening of the berries in any great degree, as the rows matured quite evenly.

Table 3 presents the yields calculated on the acre basis.

The large yield of plot 1 over that of plot 4 is due to the fact that the plants in plot 1 are one year older than the plants in plot 4. Plots 5 and 6 have practically the same yield, or at least within the limits of experimental error.

This seems to indicate that the fertilizer applied last year is sufficient to last two years. The outstanding fact in the table, however, is that a well chosen fertilizer mixture increased the crop to a point three times as great as the yield of the nearest untreated plot.

TABLE 2

Yield of blueberries on experimental plots

	JULY 17	JULY 28	AUGUST 4	AUGUST 11	AUGUST 18	TOTAL
	qls.	*qls.*	*qls.*	*qls.*	*qls.*	*q/s.*
Plot 1						
Row 1.........	2.00	2.30	1.00	0.50	None	5.80
Row 2........	4.20	2.50	1.10	0.20	None	8.00
Row 3........	5.00	5.00	1.40	0.20	None	11.60
To al.........	....	....	....	....	....	25.40
Plot 2						
Row 1........	2.50	4.16	2.00	1.00	None	9.66
Row 2........	2.80	2.65	0.90	.0.15	None	6.50
Row 3........	2.20	1.65	0.65	0.20	None	4.70
Total.........	....	....	....	....	....	20.86
Plot 3						
Row 1........	3.00	2.00	2.15	2.15	2.00	9.30
Row 2........	5.10	10.60	1.70	0.40	None	17.80
Row 3........	6.30	1.90	1.30	0.30	None	9.80
Total.........	....	....	....	....	....	36.90
Plot 4						
Row 1........	2.50	2.00	0.50	0.12	None	5.12
Row 2........	2.70	2.30	0.90	0.10	None	6.00
Row 3........	2.70	1.80	0.80	0.20	None	5.50
Total.........	....	....	....	....	....	16.62
Plot 5						
Row 1...	5.50	11.00	4.50	3.00	1.50	25.50
Row 2........	4.30	3.50	1.00	0.10	1.50	10.40
Row 3........	8.00	5.00	1.60	0.20	None	14.80
Total.........	....	....	....	....	....	50.70
Plot 6						
Row 1........	3.30	12.50	7.50	2.10	2.50	28.40
Row 2........	3.50	4.00	1.00	0.10	None	8.60
Row 3........	9.00	4.00	1.50	0.25	None	14.75
Total.........	....	....	....	....	....	51.75

The varieties represented in each row are: Row 1, Dunfee; row 2, Inman I; row 3, Inman II.

On August 10 the new growth started on all fertilized plots, in some cases being 8 inches long by August 18. This, of course, is of great advantage in setting buds for next year's crop. On August 21 none of the check plots had started new vine growth.

TABLE 3

Acre yields of blueberries on experimental plots

PLOT NUMBER	TREATMENT, 1919	TREATMENT, 1920	YIELD, 1920
			qts.
1	Nothing	Nothing	1016.0
2	250 lbs. nitrate of soda	Same as in 1919	834.4
3	250 lbs. nitrate of soda 750 lbs. acid phosphate 250 lbs. Nebraska potash	Same as in 1919	1476.0
4	Nothing	Nothing	664.8
5	170 lbs. nitrate of soda 230 lbs. dried blood 340 lbs. steamed bone 340 lbs. phosphate rock 170 lbs. Nebraska potash	Same as in 1919	2028.0
6	Same as plot 5	Nothing	2070.0

REFERENCES

(1) BECKWITH, CHARLES S. 1919 The effect of certain nitrogenous and phosphatic fertilizers on the yield of cranberries. *In* Soil Sci., v. 8, no. 6, p. 483–490.

(2) COVILLE, FREDERICK V. 1916 Directions for blueberry culture. U. S. Dept. Agr. Bul. 334.

(3) COVILLE, FREDERICK V. 1902 Blueberries. *In* Bailey's Encyclopedia of Horticulture, v. 4, p. 1889.

(4) COVILLE, FREDERICK V. 1911 Taming the wild blueberry. *In* Nat. Geogr. Mag., v. 22, p. 137–147.

(5) COVILLE, FREDERICK V. 1916 The wild blueberry tamed. *In* Nat. Geogr. Mag., v. 29, p. 535–546.

PLATE I

FIG. 1. Typical bush on plot 4, untreated.

FIG. 2. Typical bush on plot 6, treated with fertilizer in 1919.

Fig. 1.

Fig. 2.

COÖPERATIVE EXPERIMENTS FOR THE COMPOSTING OF PHOSPHATE ROCK AND SULFUR

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In Virginia the limiting factor in plant production is more often phosphorus than any of the other plant-food elements. This is proven by the widespread use of acid phosphate by the farmers of Virginia as well as by experiments conducted by the Experiment Station in different localities. Increased yields are nearly always obtained when acid phosphate is used as the carrier of phosphorus. There are other carriers of phosphorus available for use, and the relative merit of these different phosphates for agricultural purposes has been under investigation for a long time. When acid phosphate and rock phosphate have been compared, the results show that the ground rock phosphate is very slowly available and that it rarely increases the yield, at least on Virginia soils (4).

Rock phosphate, the chief source of all phosphorus fertilizers, occurs in vast deposits and it may be used with no other preparation than grinding, but the bulk of it is treated with an equal amount of sulfuric acid and converted into acid phosphate. Although this contains only about half as much phosphorus as the original rock, it is considered a much better fertilizer because the plant-food is immediately available.

A series of investigations conducted in this country and in Europe suggested to Lipman that sulfur, when composted with phosphate rock and soil, would increase the availability of the phosphorus. Lipman, McLean and Lint (5), Brown and Gwinn (2), and Ames and Richmond (1), have made investigations and have demonstrated that available phosphoric acid is produced by such treatment. For a complete bibliography see the article by McLean on the oxidation of sulfur by microörganisms (6).

In 1917 the demand for sulfuric acid for the manufacture of munitions became so great that it was feared that the supply for the manufacture of acid phosphate would be curtailed or even cut off entirely. This shortage of acid increased the cost of acid phosphate and at the same time the demand for increased crops became imperative.

In November, 1917, the National Research Council, Council of National Defense, called a meeting of agricultural workers to consider means of increasing the availability of the phosphorus in rock phosphate, which could be used as a substitute for acid phosphate. The results recorded in this paper were obtained by conducting experiments outlined at this meeting.

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The primary object of the experiment was to determine the changes that would take place when phosphate rock was composted with soil, sulfur and manure.

Materials used

Phosphate rock. The floats used was a commercial sample sold by Armour and Company of Chicago, which contained 30.99 per cent P_2O_5; apparently it was a blue rock phosphate from Tennessee.

Sulfur. Commercial ground sulfur was used.

Manure. The manure used was a mixture of horse manure and cut straw. The manure contained 76 per cent water and 0.4 per cent nitrogen.

Soil. The soil employed was obtained from the Virginia Experiment Station's plats and belonged to the Hagerstown series. It was a clay loam and had never been limed. Its composition is given in table 1.

TABLE 1

Analysis of limestone clay loam (air-dried), surface soil

	per cent
Insoluble matter (in hydrochloric acid, sp. gr. 1.115)	89.56
Potash	0.16
Soda	0.09
Lime	0.76
Magnesia	0.71
Iron oxide	0.87
Alumina	1.47
Phosphoric acid	0.07
Sulfuric acid	0.03
Water and organic matter	4.36
Humus	0.81
Nitrogen	0.136
Total potash (by J. Lawrence Smith method)	0.200
Total phosphoric acid (by fusion method)	0.133
So-called available potash (by 0.2 N nitric acid method)	0.020370
So-called available phosphoric acid (by 0.2 N nitric acid method)	0.004070

Water. Water used in keeping up the moisture content of the composts was tap water and contained 0.0014 per cent SO_3 in combination with calcium as calcium sulfate.

Composts. The composts were made up on a concrete floor. The inoculated soil, furnished by the New Jersey Agricultural Experiment Station, was first mixed with about 20 pounds of soil and was then thoroughly incorporated with 50 pounds of soil, and finally mixed with the whole portion. The sulfur was mixed thoroughly with the phosphate rock and finally the entire amount of phosphate rock, sulfur and soil was thoroughly mixed with a shovel.

A small quantity of each mixture was withdrawn and the water-holding capacity of each heap was determined. Then sufficient water was added to bring the moisture content of each heap to about 60 per cent saturation.

The·composts remained on a concrete floor under glass for the first 12 months, then all the composts were put in large boxes.   Each compost was stirred thoroughly every 10 days.   In order to hold the water content between 50 and 60 per cent of its water-holding capacity, it was necessary to cover the heaps with sacking to prevent the too rapid loss of water by evaporation. The temperature was taken at least once a day and generally twice, throughout the first year.   Apparently the temperature varied directly with the day. None of the heaps showed any evidence of heating up.   After the first year the water content was not kept within such narrow limits, the piles being allowed to dry to a considerable extent before the moisture content was brought back to 60 per cent of the water-holding capacity.

Samples were taken from composts 1A and 1B (table 2) about the first of each month.   Those from 2A and 2B were taken on the fifteenth, as the second set of composts were started 15 days later than the first.

TABLE 2

Composition of composts

	NO. 1A	NO. 1B	NO. 2A	NO. 2B
	lbs.	*lbs.*	*lbs.*	*lbs.*
Soil...	200*	400	200*	400
Ground rock phosphate.......,..............	600	600	600	600
Manure....................................			200	200
Sulfur	200		200	

* Included the inoculated soil.

EXPERIMENTAL WORK

In carrying out this work it was necessary to determine water-soluble phosphoric acid, ammonium-citrate-soluble phosphoric acid, total phosphoric acid, and the sulfur present as sulfate.   The following methods were used:

Water-soluble phosphoric acid.   Ten grams of the sample were weighed out, put on a small filter and washed with hot water until the filtrate measured 500 cc.   An aliquot was analyzed by gravimetric or volumetric methods.

Ammonium-citrate-soluble   phosphoric   acid.   The   method   followed   was suggested by J. W. Ames, and was practically the same as used by Shedd (6). Ten grams of material were treated with 100 cc. neutral ammonium-citrate solution and made up to 500 cc., and an aliquot of the filtrate was taken for analysis.

Total phosphoric acid.   Aqua regia or sulfuric acid was used as the solvent.

Sulfuric acid.   Two grams were taken and made up to 100 cc. with 1 per cent hydrochloric acid; this was shaken once every hour through the day, allowed to stand over-night, filtered and an aliquot taken for analysis.

Where the sulfur was used in the compost, the manure had not disintegrated at the end of two years.

In composts 1A and 2A both the water-soluble and ammonium-citrate-soluble phosphoric acid gradually increased (table 3). In 1A the compost of soil, ground rock phosphate and sulfur, the water-soluble phosphate increased from 0.02 per cent to 0.88 per cent, and the ammonium-citrate-soluble from 0.32 per cent to 3.10 per cent. Where manure and sulfur were used in the compost the water-soluble phosphate increased from 0.02 per cent to 1.06 per cent, and the ammonium-citrate-soluble from 0.39 to 3.35 per cent. In

TABLE 3

Water-soluble and ammonium-citrate-soluble phosphoric acid (P_2O_5) found in the composts

DATE OF TAKING SAMPLE	NO. 1A. SOIL, GROUND ROCK PHOSPHATE AND SULFUR		NO. 1B. SOIL AND GROUND ROCK PHOSPHATE		NO. 2A. SOIL, MANURE, GROUND ROCK, PHOSPHATE AND SULFUR		NO. 2B. SOIL, MANURE, GROUND ROCK PHOSPHATE	
	Water-soluble	Ammonium-citrate-soluble	Water-soluble	Ammonium-citrate-soluble	Water-soluble	Ammonium-citrate-soluble	Water-soluble	Ammonium-citrate-soluble
1918	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
January......	0.02	0.32	Trace	0.30	0.02	0.39	Trace	0.39
February.....	0.03	0.35	Trace	0.30	0.02	0.38	Trace	0.39
March.......	0.03	0.36	Trace	0.31	0.02	0.36	Trace	0.37
April........	0.10	0.37	Trace	0.34	0.40	0.96	Trace	0.38
May.........	0.30	1.06	Trace	0.29	0.56	1.37	Trace	0.35
June.........	0.70	1.45	Trace	0.30	0.90	1.88	Trace	0.36
July.........	0.78	1.63	Trace	0.30	0.80	2.18	Trace	0.32
August.......	0.77	1.88	Trace	0.30	0.77	2.28	Trace	0.31
September....	0.78	2.00	Trace	0.32	0.82	2.45	Trace	0.31
October......	0.79	2.30	Trace	0.34	0.83	2.85	Trace	0.32
November....	0.80	2.42	Trace	0.32	0.90	2.81	Trace	0.37
December....	0.77	2.45	Trace	0.32	0.92	2.87	Trace	0.31
1919								
January......	0.82	2.42	Trace	0.30	0.87	2.75	Trace	0.33
March.......	0.54	2.42	Trace		0.66	2.64	Trace	
June.........	0.76	2.62	Trace	0.57	0.91	3.06	Trace	0.45
September....	0.77	2.79	Trace	0.60	1.06	3.35	Trace	0.33
1920								
January......	0.88	3.01	0.02	0.67	0.56	3.12	0.02	0.31

this instance the maximum percentage occurred in September, 1919, and dropped at the next analysis in January.

In 1B and 2B both the water-soluble and ammonium-citrate-soluble phosphoric acid remained practically constant throughout the whole experiment. In 1B, the compost without manure, the ammonium-citrate-soluble phosphoric acid increased 0.37 per cent in the last two determinations.

The increase in the percentage of sulfur as SO_3 goes hand in hand with the increase of available phosphorus, and in fact slightly precedes the gain of the latter, as shown in table 4.

TABLE 4

Sulfur present as SO₃

DATE OF TAKING SAMPLE	NO. 1A. SOIL, GROUND ROCK PHOSPHATE AND SULFUR	NO. 1B. SOIL AND GROUND ROCK PHOSPHATE	NO. 2A. SOIL, MANURE, GROUND ROCK PHOSPHATE AND SULFUR	NO. 2B. SOIL, MANURE AND GROUND ROCK PHOSPHATE
1918	*per cent*	*per cent*	*per cent*	*per cent*
January............	1.11	1.01	1.10	1.11
February..........	1.05	1.03	1.09	1.16
March.............	1.62	0.96	2.45	1.06
April..............	2.43	0.99	2.94	1.11
May...............	3.32	1.14	3.40	1.11
June..............	4.17	1.24	3.78	1.52
July..............	4.36	1.01	6.20	1.43
August............	7.12	1.50	6.35	1.70
September.........	7.81	1.45	8.00	1.70
October...........	8.46	1.40	8.52	1.53
November.........	8.75	1.40	6.52	1.52
December.........	7.13	2.20	6.61	1.51
1919				
January............	7.73	2.37	7.13	1.38
March.............	8.40		8.16	
June..............	9.04	3.06	8.66	1.68
September.........	9.21	3.50	8.15	1.98
1920				
January............	8.92	3.57	7.70	1.92

TABLE 5

Comparison of the availability of phosphoric acid

	NO. 1A	NO. 1B	NO. 2A	NO. 2B
	per cent	*per cent*	*per cent*	*per cent*
At the beginning:				
Total P₂O₅............................	19.13	18.81	18.33	18.11
Water-soluble P₂O₅.....................	0.02		0.02	
Ammonium-citrate-soluble P₂O₅...........	0.32	0.30	0.39	0.39
Total P₂O₅ available....................	1.67	1.59	2.13	2.15
December, 1919 (end 1 year):				
Total P₂O₅............................	16.35	18.81	15.52	18.81
Water-soluble P₂O₅.....................	0.77		0.92	
Ammonium-citrate-soluble P₂O₅...........	2.45	0.32	2.87	0.31
Total P₂O₅ available....................	14.98	1.70	18.48	1.64
January 8, 1920 (end second year)				
Total P₂O₅............................	16.30	18.44	16.16	18.36
Water-soluble P₂O₅.....................	0.88	0.02	1.06	
Ammonium-citrate-soluble P₂O₅...........	3.01	0.67	3.35	0.33
Total P₂O₅ available....................	18.47	3.63	20.73	1.80

The greatest increase in sulfate was found where soil, sulfur and rock phosphate were composted. The amount was greater than in compost 2A, where manure was present. At the beginning, the addition of manure apparently increased the sulfate formation, but by the end of the year the compost without the manure contained the higher percentage of sulfates.

Where soil and ground rock phosphate were composted the percentage of sulfates remained more or less constant for a year, and then a slight increase occurred. The increase was from 1.01 per cent to 3.57 per cent. Where manure was added the percentage of sulfates did not increase very much the first year and the total increase was not as great as that in compost 1B.

In compost 2A where sulfur, manure, soil and rock phosphate were used, the available phosphoric acid increased from 2.13 per cent to 20.73 per cent (table 5). The next largest increase was shown in compost 1A which contained phosphate rock, soil and sulfur. The available phosphoric acid increased from 1.67 to 18.47 per cent.

In the composts where no sulfur was added, there was practically no increase of available phosphoric acid, and in compost 2B where manure was added, there was a loss of available phosphoric acid.

Supplementary Experiment No. 1. A supplementary experiment was undertaken which had for its object the study of the changes that would occur in the nitrogen content of manure, where sulfur and rock phosphate were used as preservatives. The composts were prepared and analyzed substantially as those discussed above, and had the following composition:

	COMPOST A	COMPOST B
	lbs. .	lbs.
Manure...	500	500
Floats...	5	5
Sulfur...	1¾	

During the first year these composts were stirred thoroughly once every 10 days and watered whenever the moisture content was low. Samples were taken about the fifteenth of the month, air-dried, ground and analyzed. The ammonia, nitrate and total nitrogen content of the compost were determined in the original samples and at intervals running over a period of two years.

The results given in table 6 show that the percentage of total nitrogen in compost A remained practically constant. In compost B, where sulfur was absent, the nitrogen percentage showed an increase due to the decomposition of the organic matter.

The nitrate content of compost A remained constant, the ammonia content increased from 0.015 per cent to as high as 0.424 per cent of nitrogen, gradually increasing for six months and then gradually dropping until at the end of two years, when only 0.098 per cent of nitrogen was present.

In compost B the nitrogen as nitrates increased from 0.045 to 0.526 per cent, the high mark being reached at the end of one year. At the end of

TABLE 6

Nitrogen content of dry samples

DATE	TOTAL NITROGEN		NITROGEN AS NITRATES		NITROGEN AS AMMONIA	
	A	B	A	B	A	B
1918	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Sample No. 1............	1.657	1.756	0.067	0.045	0.015	0.030
February................	1.812	1.980	0.038	0.068	0.091	0.015
April 14................	1.904	2.395	0.007	0.076	0.202	0.015
May 15................	1.825	2.425	0.045	0.407	0.328	0.030
June 17................	1.968	2.370	0.045	0.414	0.415	0.030
July 15................	1.713	2.393	0.000	0.498	0.424	0.045
August 15..............	1.814	2.366	0.030	0.487	0.404	0.045
September 16............	1.803	2.332	0.000	0.501	0.327	0.030
October 15..............	1.745	2.479	0.030	0.487	0.304	0.015
November 15............	1.632	2.329	0.052	0.473	0.224	0.030
December 11.............	1.758	2.355	0.067	0.526	0.252	0.015
1919						
January 11..............	1.685	2.441	0.045	0.442	0.260	0.015
June 2.................	1.547	2.341	0.112	0.416	0.142	0.015
September 2.............	1.577	2.287	0.044	0.476	0.118	0.022
1920						
January 8..............	1.609	2.190	0.053	0.391	0.098	0.008

A = Manure, floats and sulfur; B = manure and floats.

TABLE 7

Comparison of nitrogen determinations

.	A. MANURE, ROCK PHOSPHATE AND SULFUR		B. MANURE AND ROCK PHOSPHATE	
	February, 1918 beginning	January, 1920 end	February, 1918 beginning	January, 1920 end
Weight of mixture..............	506⅔ lbs.	200 lbs.	505 lbs.	109 lbs.
Per cent of water..............	76.46	66.48	77.03	55.09
Amount of dry matter..........	119.27 lbs.	67.04 lbs.	116.00 lbs.	48.95 lbs.
Per cent of nitrogen............	0.398	0.5388	0.410	0.9844
Total amount of nitrogen........	2.012 lbs.	1.0776 lbs.	2.072 lbs.	1.073 lbs.
Dry matter lost...............	52.23 lbs., or 43.79 per cent		67.05 lbs. or 57.80 per cent	
Nitrogen lost..................	0.934 lbs., or 46.66 per cent		0.999 lbs. or 48.21 per cent	
Amount of nitrogen as ammonia..	0.047 lbs. gain		0.031 lbs. loss	
Amount of nitrogen as nitrate....	0.046 lbs. loss		0.148 lbs. gain	

two years the nitrate content had dropped to 0.391 per cent of nitrogen. The ammoniacal nitrogen remained practically constant.

Compost A, where sulfur was present, did not lose as much organic matter by the various decomposing agents as compost B. The difference in weight

TABLE 8

Sulfofying power of certain Virginia soils

SOIL	SULFUR AS SO₄ MAY 2	SULFUR AS SO₄ MAY 11	SULFUR AS SO₄	AMOUNT OF SULFUR ADDED	SULFUR OXIDIZED
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*
Loudon County					
Penn gravelly loam..................	0.42	7.04	6.62	13.33	49.66
Penn stony loam....................	1.27	1.39	0.12	13.33	0.90
Penn loam..........................	2.78	4.79	2.01	13.33	15.08
Cecil mica loam....................	0.87	3.63	2.76	13.33	20.71
Penn clay..........................	1.11	3.92	2.81	13.33	21.08
Cecil clay.........................	3.75	4.60	0.85	13.33	6.38
Cecil loam.........................	2.22	4.74	2.52	13.33	18.90
Cecil silt loam....................	1.67	6.36	4.69	13.33	35.18
Iridell clay loam..................	1.39	5.16	3.77	13.33	28.28
Loudon sandy loam.................	1.67	6.36	4.69	13.33	35.18
Frankstown gravelly loam...........	0.73	4.95	4.22	13.33	31.66
Fredericksburg stony loam..........	1.51	4.17	4.66	13.33	19.95
Fredericksburg silt loam...........	1.67	10.32	8.65	13.33	64.89
DeKalb silt loam...................	3.95	6.06	2.11	13.33	15.83
DeKalb gravelly loam...............	2.36	3.43	1.07	13.33	8.03
Hagerstown stony clay loam.........	3.33	7.04	3.71	13.33	27.83
Hagerstown clay loam..............	2.22	4.44	2.22	13.33	16.65
Berks shale loam...................	2.22	4.76	2.54	13.33	19.05
Berks silt loam....................	2.22	4.94	2.72	13.33	20.41
Bedford County					
Murrill clay loam..................	0.86	2.16	1.30	13.33	9.75
Murrill fine sandy loam............	0.81	2.25	1.44	13.33	10.80
Campbell county					
Iridell fine sandy loam............	0.69	2.22	1.53	13.33	11.48
Louisa fine sandy loam	0.50	2.68	2.18	13.33	16.35
York loam.........................	0.88	2.22	1.34	13.33	10.05
York free sandy loam..............	0.73	2.25	1.52	13.33	11.40
Louisa loam.......................	0.75	2.22	1.47	13.33	11.03
Appomattox County					
Cecil sandy loam...................	0.72	2.00	1.28	13.33	9.60
Cecil loam.........................	1.75	2.36	0.61	13.33	4.58
Iridell clay loam..................	1.11	2.42	1.31	13.33	9.83
Cecil clay.........................	0.91	2.50	1.59	13.33	11.93
Prince Edward County					
Worsham sandy loam...'............	0.85	2.36	1.51	13.33	11.33
Durham sandy loam................	0.81	2.11	1.30	13.33	9.75
Iridell clay loam..................	0.70	1.75	1.05	13.33	7.88
Norfolk County					
Leonardstown loam.................	0.82	2.22	1.40	13.33	10.50
James City County					
Leonardstown loam.................	0.83	1.75	0.92	13.33	6.90
Norfolk fine sandy loam............	0.65	2.36	1.71	13.33	12.83
Albemarle County					
Cecil sandy loam...................	0.77	2.61	1.84	13.33	13.80
Ceci! loam........................	0.73	2.22	1.49	13.33	11.18
Cecil clay.........................	0.72	1.83	1.11	13.33	8.33

of the compost as well as the total amount of nitrogen present at the end of the experiment proved this. When the bacteria were counted, compost B, without sulfur, showed the presence of from three to four times as many bacteria as compost A.

When manure and ground rock phosphate were composted the nitrogen lost amounted to 48.21 per cent (table 7). The loss of nitrogen as ammonia was 0.031 pound, while there was a gain of 0.138 pound of nitrogen as nitrate. Where sulfur was used in the compost a gain of 0.047 pound of ammoniacal nitrogen was shown. The loss of nitrate nitrogen in composting was 0.046 pound. No increase of available phosphoric acid was found in either of the composts.

THE SULFOFYING POWER OF CERTAIN TYPES OF VIRGINIA SOIL

Table 8 gives the sulfofying power of some of the soil types of Virginia. The determinations are made by Mr. T. J. Murray, who followed Brown's (3) method. Sodium sulfate was the salt oxidized.

DISCUSSION

In compost 1A, which contained sulfur, soil and rock phosphate, 10.89 per cent of the total phosphoric acid was available after 7 months, 14.98 per cent after 12 months, and 18.47 per cent after 2 years.

In compost 2A, which contained manure, sulfur, soil and phosphate rock, a larger percentage of the total phosphoric acid was made available than in compost 1A without manure. At the end of 7 months 14.69 per cent was available, at the end of one year 18.48 per cent, and after 2 years 19.31 per cent.

Our results agree with those obtained by Brown and Gwinn (2) which show that more available phosphoric acid is produced where manure is included in the compost.

Shedd's results (7) with soil, rock phosphate and sulfur, in the same proportions used by us, agree fairly well for the first 7 months of the experiment, but where he used sulfur, soil, rock phosphate and manure in different proportions, there is a lack of agreement. Our results do not show as great sulfur oxidation with the accompanying high percentage of available phosphoric acid.

In composts 1A and 2A, over 10 per cent of the phosphoric acid was made available in 7 months. Where no sulfur was used in composts 1B and 2B, the available phosphoric acid remained practically constant throughout the experiment. There was a slight increase in the available phosphoric acid at the end of 2 years in compost 1B which contained soil and rock phosphate.

Manure did not increase the available phosphoric acid in compost 2B after 2 years, and the analyses showed less available phosphoric acid at the end of the experiment than was present at the beginning.

Where 5 pounds of rock phosphate was added to 500 pounds of manure, 57.80 per cent of the dry matter and 48.21 per cent of nitrogen were lost in 2 years.

Where 5 pounds of rock phosphate, and 1⅔ pounds of sulfur were added to 500 pounds of manure, the decomposition was less. The losses were as follows: dry matter 43.79 per cent; nitrogen 46.44 per cent.

In compost A, with sulfur, ammoniacal nitrogen was produced whereas we do not find an increase of nitrates.

In compost B, without sulfur, nitric nitrogen was produced. There was a decrease in the ammoniacal nitrogen.

SUMMARY

1. The addition of sulfur to a compost of soil and rock phosphate increased the availability of phosphoric acid, but not to the same extent as when manure was added to a compost of soil, rock phosphate and sulfur.

2. In compost 1B and 2B, without sulfur, neither compost showed any appreciable increase in the availability of the phosphoric acid.

3. Sulfur oxidation preceded the increase of available phosphoric acid.

4. The addition of phosphate to manure slowed up the fermentation and there was a loss of only 57.80 per cent of dry matter and 48.21 per cent of nitrogen in 2 years. At the same time there is an increase of 0.138 pound of nitrate nitrogen and a loss of 0.031 pound of ammoniacal nitrogen.

5. The addition of sulfur and phosphate to manure checked the fermentation to a greater extent than the phosphate alone. There was a loss of only 43.77 per cent of dry matter and 46.44 per cent of nitrogen in 2 years. But here the increase in ammoniacal nitrogen was balanced by the loss in the nitrate nitrogen.

6. Upon the addition of rock phosphate to manure a large quantity of nitric nitrogen was formed. When sulfur also was added there was no nitrate formation but the ammonia content was greatly increased.

7. All of the Virginia soils tested had some sulfofying power, but there was a very great variation among the different soils.

8. The majority of the Virginia soils oxidized less sulfur than the soils tested by Shedd (7) of Kentucky and Brown and Kellogg (3) of Iowa.

9. The results of composting soil, sulfur, ground phosphate · rock and manure, under the conditions suggested by the plan as outlined, do not in our opinion warrant the farmers of Virginia conducting experiments along similar lines, because the formation of available phosphoric acid is too slow to meet their needs. Besides, the farmer cannot be expected to keep the water-holding capacity of his composts up to the desired amount, and he is not likely to spade the composts every 10 or more days so that the proper oxidation would occur. Probably some conditions might arise or different propor-

tions of soil, rock phosphate, and sulfur be suggested, or a better starter or inoculating material might be used, which would give higher sulfur oxidation and thereby produce more available phosphoric acid than is possible under the conditions as outlined in this experiment.

REFERENCES

(1) AMES, J. W., AND RICHMOND, T. E. 1918 Sulfofication in relation to nitrogen transformations. *In* Soil Sci., v. 5, no. 4, p. 311–321.

(2) BROWN, P. E., AND GWINN, A. R. 1917 Effect of sulfur and manure on availability of rock phosphate in soil. Iowa Agr. Exp. Sta. Res. Bul. 43.

(3) BROWN, P. E., AND KELLOGG, E. H. 1914 Sulfofication in soils. Iowa Agr. Exp. Sta. Res. Bul. 18.

(4) ELLETT, W. B., AND INGHAM, A. A. 1917 Pot experiments to determine primarily the availability of phosphoric acid in Thomas slag phosphates in comparison with other phosphates. Va. Agr. Exp. Sta. Tech. Bul. 16.

(5) LIPMAN, JACOB G., McLEAN, HARRY C., AND LINT, H. C. 1916 Sulfur oxidation in soils and its effect on the availability of mineral phosphates. *In* Soil Sci., v. 6, no. 4, p. 499–538.

(6) McLEAN, HARRY C. 1918 The oxidation of sulfur by microorganisms in its relation to the availability of phosphates. *In* Soil Sci., v. 5, no. 4, p. 251–290.

(7) SHEDD, O. M. 1919 Effect of oxidation of sulfur in soils on the availability of rock phosphate and on nitrification. *In* Jour. Agr. Res., v. 18, no. 6, p. 329–345.

THE INFLUENCE OF INITIAL REACTION ON THE OXIDATION OF SULFUR AND THE FORMATION OF AVAILABLE PHOSPHATES[1]

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Earlier experiments on the production of soluble phosphates through the oxidation of sulfur have indicated certain more or less well defined steps both in the oxidation of the sulfur and in the formation of soluble phosphates. It would seem that the activities of the sulfur-oxidizing bacteria gain in intensity when the reaction of the medium becomes acid beyond a certain point. For this reason it has seemed advisable to determine whether by adjusting the reaction of the medium the processes of sulfur oxidation might be expedited. Accordingly, mixtures were made up of greenhouse soil, ground phosphate rock and flowers of sulfur. The proportions used were:

	grams
Soil	100
Tennessee rock phosphate, containing 31.12 per cent of total phosphoric acid	15
Flowers of sulfur	5

The mixtures were kept in tumblers with a moisture content of about 38 per cent. As shown in table 1, additions were made to the mixtures of different amounts of 0.1 N sulfuric acid. The largest amount of acid added to any of the tumblers was 45 cc. and the smallest 12 cc. No acid was added to the mixture used as a check.

When the experiment was begun on March 16, 1920, the initial hydrogen-ion exponent, the pH of Sörensen, ranged from 5.4 in mixture 16 to 4.7 in no. 15. In order to provide suitable inoculation there was added to each mixture 5 cc. of infusion prepared by shaking 100 gm. of material from an old sulfur-phosphate compost with 500 cc. of distilled water. It may be noted, in this connection, that these composts were originally made up in the fall of 1916 and that by the spring of 1920 the hydrogen-ion exponent as expressed in pH values was 1.6. Water extract for the determination of the hydrogen-ion concentration was prepared according to the method of Gillespie (2). The hydrogen-ion concentrations of the extract were determined by the colorimetric method, using the sulfonephthalein series of indicators as recommended by Clark and Lubs (1).

Aside from the hydrogen-ion concentration determinations made from time to time, tests were made also for sulfates as a check on the amount of sulfuric acid produced. The soluble phosphates were determined by the method of the Official Agricultural Chemists. During the progress of the experiment the tumblers containing the different mixtures were kept covered with petri dishes. The weight of each tumbler and contents were marked on the petri dishes and the optimum amount of moisture was maintained by restoring the water lost by evaporation. Proper allowance was made for the small quantities of material withdrawn for the different determinations.

The progress of sulfur oxidation can be readily traced from the data given in table 2.

TABLE 1

Treatment of soil samples studied

MIXTURE NUMBER	pH	0.1 N H_2SO_4	H_2O
		cc.	cc.
1	5.0	12.0	29.0
2	5.0	14.4	26.6
3	5.1	16.8	24.2
4	5.1	19.2	21.8
5	5.0	21.4	19.6
6	5.0	24.0	17.0
7	5.0	26.4	14.6
8	5.0	28.8	12.2
9	5.0	31.2	9.8
10	5.0	33.6	7.4
11	4.8	36.0	5.0
12	4.9	38.4	2.6
13	4.8	40.8	0.2
14	4.9	43 2	0.0
15	4.7	45.0	0.0
16	5.4	0.0	41.0

It is quite evident from the data recorded in the table that the initial reaction did not appreciably affect the rate of sulfur oxidation, nor the rate of the formation of soluble phosphates. Within one week after the beginning of the experiment the pH was as low as 3.6 in mixture 13. At the end of the third week the pH was below 3.0 in all of the mixtures. It may be of interest to point out in this connection that in mixture 13 there was no calcium phosphate added and that, for this reason, there was a greater accumulation of acidity in the material than in any of the other mixtures where the calcium of the tricalcic phosphate served in part to neutralize the free sulfuric acid. At the end of the twelfth week the pH was below 2.0 in tumblers 6, 8 and 12, as well as in tumbler 13. Beyond that the increase in acidity was relatively slight.

It will be observed that the formation of available phosphate showed a very marked increase between the end of the second and the end of the third

TABLE 2

Sulfur oxidation as indicated by the availability of phosphate

NUMBER OF CULTURE	END OF FIRST WEEK		END OF SECOND WEEK		END OF THIRD WEEK		END OF FOURTH WEEK		END OF FIFTH WEEK		END OF SIXTH WEEK		END OF EIGHTH WEEK	
	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*
1	4.2	3.75	3.0	4.25	2.8	13.26	2.8	14.72	2.6	15.3	2.4	23.2	2.6	31.8
2	4.1	3.90	3.0	4.25	2.8	14.84	2.8	15.40	2.6	17.1	2.4	26.2	2.4	32.7
3	4.1	4.01	3.0	5.00	2.8	15.02	2.8	15.50	2.4	19.1	2.4	28.4	2.4	33.7
4	4.1	4.01	3.0	6.78	2.8	11.46	2.8	13.30	2.6	18.7	2.4	27.9	2.6	35.2
5	4.0	4.21	3.0	6.76	2.8	12.52	2.8	13.30	2.4	19.4	2.3	30.0	2.4	36.3
6	4.0	4.21	3.0	7.80	2.8	15.94	2.8	15.60	2.4		2.4	26.8	2.2	31.4
7	4.1		3.0	7.80	2.8	14.72	2.8	15.40	2.4	19.5	2.4	26.3	2.4	32.0
8	4.1	4.60	3.0	7.80	2.8	17.64	2.8	17.30	2.4	19.4	2.4	27.0	2.2	32.6
9	4.0	5.23	3.0	7.55	2.8	15.40	2.8	15.60	2.4	19.3	2.4	27.2	2.2	33.2
10	4.0	4.60	3.4	4.74	2.8	17.60	2.8	19.14	2.4	21.3	2.3	30.6	2.2	36.7
11	4.1	4.01	3.6	4.60	2.8	14.64	2.8	14.64	2.6	15.3	2.4	24.2	2.4	32.9
12	4.1	4.50	3.0	4.90	2.8	15.64	2.8	16.56	2.4	20.4	2.4	27.3	2.1	34.2
13	3.6		3.4		2.4		2.4		2.4		2.0		2.0	
14	4.0	5.23	3.8	5.20	2.8	15.02	3.0	15.44	2.8	21.1	2.6	29.0	2.4	35.6
15	4.0	5.30	3.2	4.47	2.8	15.10	3.0	15.02	2.6	15.4	2.6	26.8	2.6	31.6
16	4.4	3.75	3.0	4.38	2.8	14.90	2.8	15.44	2.6	19.1	2.4	27.2	2.6	33.4

NUMBER OF CULTURE	END OF NINTH WEEK		END OF TENTH WEEK		END OF TWELFTH WEEK		END OF FOURTEENTH WEEK		END OF SIXTEENTH WEEK		END OF EIGHTEENTH WEEK		END OF TWENTIETH WEEK	
	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*	pH	Available P *per cent*
1	2.4	35.2	2.3	42.4	2.2	59.8	2.2	59.0	2.2	66.9	2.1	76.3	1.9	83.6
2	2.4	36.1	2.3	43.0	2.2	59.6	2.2	60.1	2.2	68.4	2.1	77.2	2.0	83.9
3	2.4	36.2	2.3	44.1	2.2	60.1	2.3	59.4	2.3	64.6	2.2	73.5	2.0	84.9
4	2.6	36.4	2.2	45.2	2.2	60.3	2.3	61.0	2.3	65.0	2.0	74.9	2.0	84.9
5	2.4	35.9	2.2	44.1	2.1	63.4	2.1	62.7	2.0	69.0	2.0	75.3	1.8	86.3
6	2.2	36.2	2.0	43.7	1.9	65.4	1.9	67.4	1.9	69.7	1.9	78.4	1.9	84.9
7	2.4	35.7	2.3	42.8	2.3	62.3	2.3	60.4	2.3	63.6	2.1	76.1	2.0	82.9
8	2.2	36.6	2.1	43.9	1.9	65.0	1.8	68.2	1.8	71.2	1.8	79.9	1.8	86.8
9	2.2	37.0	2.1	44.2	2.0	66.1	1.9	69.1	1.9	73.0	1.9	80.1	1.9	84.9
10	2.2	37.7	2.1	45.0	2.0	64.9	2.0	60.2	2.0	68.2	1.9	79.4	1.9	85.2
11	2.4	35.3	2.2	43.7	2.2	59.8	2.3	58.7	2.2	63.4	2.0	74.2	2.0	84.9
12	2.1	36.8	2.0	43.8	1.9	60.1	1.9	64.6	1.9	69.8	1.8	79.9	1.8	85.3
13	2.0		1.6		1.4		1.4							
14	2.4	35.2	2.2	42.6	2.0	64.2	2.0	63.7	2.0	67.9	1.9	78.4	1.9	84.4
15	2.6	33.8	2.2	45.0	2.2	60.1	2.0	60.2	2.1	65.8	2.0	77.3	2.0	83.9
16	2.4	34.9	2.3	44.2	2.2	59.9	2.3	61.0	2.2	66.6	2.0	77.6	2.0	85.1

week. *For instance, in mixtures 8 and 10 the proportion of available phos-phates was well above 17 per cent.   At the end of the fifth week the increases in the amounts of available phosphoric acid over those found at the end of the third week were not large.   On the other hand, a very marked increase occurred between the end of the fifth and the end of the sixth week.   For instance, in mixtures 5 and 10 there were found at that time 30 per cent of the phos-*
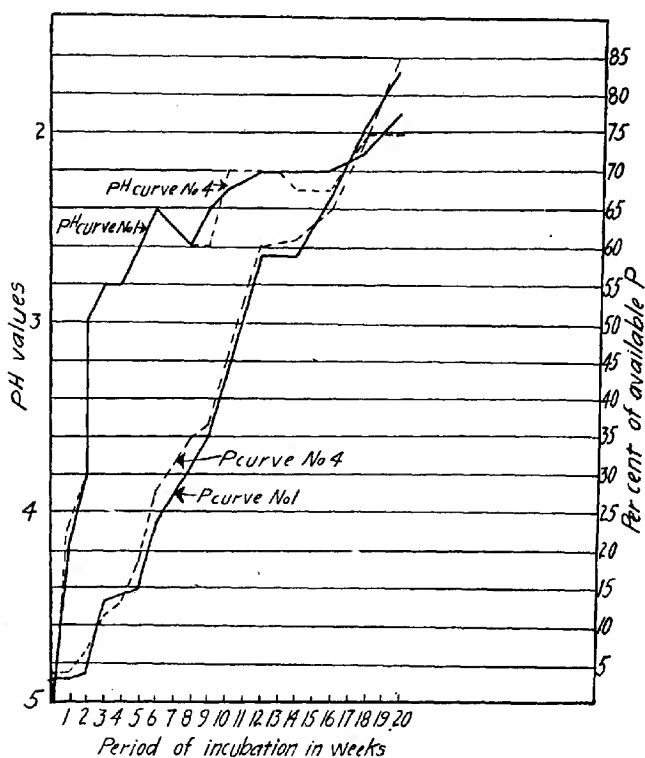
FIG. 1. CURVES OF HYDROGEN-ION CONCENTRATION AND PER CENT OF AVAILABLE PHOS-PHORIC ACID AS P IN SAMPLES 1 AND 4

phoric acid in an available form.   Progressive and marked increases occurred at the end of the eighth, ninth and tenth weeks. A very marked increase occurred again between the end of the tenth and that of the twelfth week. This continued until the end of the twentieth week when the proportion of available phosphoric acid was above 82 per cent in all cases and in at least two instances well above 86 per cent.

In attempting to interpret the data just given one should bear in mind that the pH determinations record the intensity of the acid rather than the quantity of it. As time went on the quantity of sulfuric acid or of acid sulfates accumulating in the mixtures gradually increased. There was, therefore, a gradu-
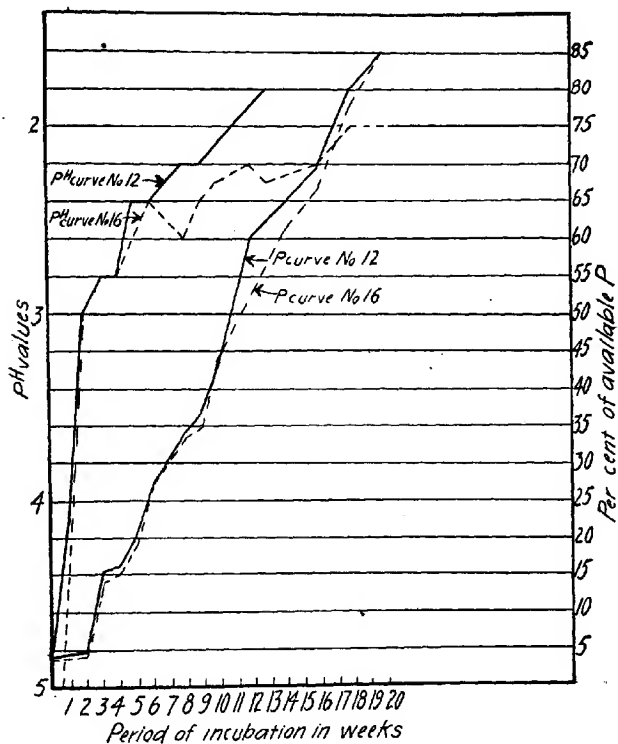
FIG. 2. CURVES OF HYDROGEN-ION CONCENTRATION AND PER CENT OF AVAILABLE PHOS-PHORIC ACID AS P IN SAMPLES 12 AND 16

ally increasing quantity of acid material available for reacting with the tricalcic phosphate. Figures 1 and 2 show in a graphic way the progressive changes in mixtures 1, 4, 12 and 16. It is expected that other data now available, and confirming the results recorded in this paper, will be made ready for publication in the near future.

CONCLUSIONS

This set of experiments shows no advantage in starting with a relatively high hydrogen-ion concentration through additions of sulfuric acid. On the other hand, there is evidence that such advantage may be had in mixtures of a different composition. The data in question will be reported at a later date.

REFERENCES

(1) CLARK, W. M., AND LUBS, H. A. 1917 The colorimetric determination of hydrogen-ion concentration, and its application in bacteriology. *In* Jour. Bact., v. 2, p. 1–34.
(2) GILLESPIE L. J. 1916 The reaction of soil and measurements of hydrogen-ion concentration. *In* Jour. Wash. Acad. Sci., v. 6, p. 7–16.

THE DETERMINATION OF NITRITES AND NITRATES IN PLANT TISSUE[1]

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INTRODUCTION

On account of the complex nature of plant juices the determination of the nitrate nitrogen therein is beset with many possibilities of error. In most of the work done upon the nitrate content of plants the Schloesing method and its modifications have been used. Some investigators have criticized this method and have recommended other methods for the purpose. Since the determination of nitrates in plants proved of great importance in the solution of a problem under investigation, it seemed necessary to make a careful study of certain methods for nitrates in order to determine their applicability to plant tissue.

Without attempting a complete discussion of the literature, the methods employed for nitrates in plant tissue, or in analogous substances will be briefly considered.

In studies of the nitrate content of plant tissue Schulze (11), Nedokvochayev (8), Woo (13) and others used various modifications of Schloesing's (12, p 456) method. This method is based upon the measurement of nitric oxide gas which is liberated when nitric acid is heated with ferrous chloride and hydrochloric acid. Krog and Sebelien (7) claim that the Schloesing method gives low results in the presence of carbohydrates and other organic substances. They obtained much more satisfactory results with the "nitron" method. In this work Krog and Sebelien used both water and alcohol (2 to 1) in making extracts of green plants. The "nitron" method devised by Busch (3) is based upon the formation of an insoluble compound by the interaction of nitron (diphenyl-endo-anilo-hydro-triazole) and nitric acid.

Caron (4) obtained good results in the determination of nitrates in urine by the colorimetric method. He determined the intensity of color produced when a nitrate solution is treated with diphenylamine and sulfuric acid.

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Another method for the determination of nitrates, known as Devarda's method, is based upon the reduction of nitric acid to ammonia by means of Devarda's alloy (50 parts copper, 45 parts aluminum and 5 parts zinc) in alkaline solution (12, p. 454). This method somewhat modified in detail is recommended by Allen (1), and others for nitrate nitrogen in soils and by Davisson (5) for nitrates in "soil and physiological extracts."

In the Ulsch-Street method (2) nitric acid is reduced with iron and dilute sulfuric acid and the ammonia obtained thereby distilled from magnesia.

Zeller (14) proposed a method for determining nitrates and nitrites in the presence of much organic matter. For nitrates the Ulsch-Street method was used, while nitrites were determined by measuring the amount of ammonia decomposed by nitrous acid according to the following reaction:

$$NH_3 + HNO_2 = N_2 + 2H_2O$$
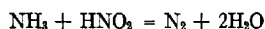
In the determination of the latter he used a known quantity of ammonium chloride and evaporated the unknown solution to a small volume. The solution was then diluted and distilled from magnesia into standard acid. The nitrite was calculated by loss of ammonia. He obtained good results with peptones, soil and plant decoctions.

<div style="text-align:center">EXPERIMENTAL</div>

<div style="text-align:center">I. Nitrates</div>

The colorimetric method of Caron proved unsatisfactory, as the blue color obtained by treating plant extracts with diphenylamine faded so rapidly that accurate results were impossible. The determination of nitrates in plants by finding the difference between the nitrogen obtained by the Kjeldahl method modified to include nitrate and the Kjeldahl-Gunning-Arnold method also was unsatisfactory, since appreciable amounts of nitrate were apparently reduced without zinc and salicylic acid.

The "nitron" method was next tried. The results are shown in table 1. Many substances give somewhat insoluble compounds with nitron and therefore the method must be used with caution. Among the substances likely to be present in plants that will give high results are nitrites and oxalates. While the rather meager data given above show fair results, the unexpectedly high nitrate content made it advisable to test the accuracy of the method further. On account of the variety of substances which may be present in plants that may affect the accuracy of this method, it was decided to compare it with another method based on a different principle rather than to attempt to eliminate troublesome compounds which may be present.

Consequently, the Devarda method was studied. Allen (1) claimed that this method was accurate for soils when 0.1 N sodium hydroxide was used in reduction instead of the more concentrated alkali originally recommended. He boiled the solution for $\frac{1}{2}$ hour before addition of alkali in order to get rid of

the "albuminoid" ammonia. Davisson showed that boiling ½ hour did not get rid of all the ammonia and he suggested previous precipitation with Stutzer's reagent to get rid of "protein like substances." However, this would not free the solution from certain amino acids (arginine and cystin), or bases (e.g. guanidine) which may be present in both soils and plants, and which would cause an error in determination by this method. A simpler and better method might be to run a control without alloy on each extract. The possible difficulties here might be:

1. That the compounds in plants yielding ammonia on boiling with sodium hydroxide may be very sensitive to a change in concentration of alkali which would be caused by the reaction of the alkali with the alloy.

2. That the alloy might act catalytically to break down some compounds which would yield ammonia.

The possibility of error from these sources was accordingly studied and the following experiments undertaken. Two proteins, lactalbumin and arachin,

TABLE 1

Nitron method for nitrates

SUBSTANCE	MODIFIED KJELDAHL METHOD	NITRON METHOD
	Nitrate nitrogen	Nitrate nitrogen
	mgm.	*mgm.*
Pure NaNO₃ solution....................................	3.68	3.77
Pure NaNO₃ solution + nitrate-free plant extract...........	3.68	3.88
Plant extract containing nitrate + 7.35 mgm. N from NaNO₃.		9.46
Nitrate nitrogen in plant extract by difference...............		1.90
Nitrate nitrogen extract by direct determination.............		1.75

both rich in arginine, were hydrolyzed by boiling for 48 hours in 20 per cent hydrochloric acid under a reflux condenser. They were allowed to cool, partially neutralized with sodium hydroxide and then made alkaline with sodium carbonate. The humin was then filtered off. The alkaline solutions were next aerated 8 hours to get rid of ammonia. Amino acids thus obtained in amounts corresponding to 0.2 gm. of protein were treated as shown in table 2.

Taken as a whole these results show that no appreciable error is caused by the difference in concentration of alkali caused by action of alloy, nor does the presence or absence of alloy affect the amount of ammonia evolved. The amount of amino acids used equals the amount in extract from several grams of flowering soybean plants grown in sand containing nitrate.

An experiment was next undertaken to determine the effect of amino acids, asparagin, and variation in the concentration of alkali on the determination of nitrates by Devarda's method (table 3).

It is again seen that a change in concentration of alkali due to the alloy's action does not affect the accuracy of the determination, and that the presence

TABLE 2

Effect of different concentrations of alkali and of alloy on NH₃ obtained by boiling amino acids with NaOH in Devarda's method for nitrates

TREATMENT	N/28 NaOH	N
Amino acids* + 250 cc. H₂O+	cc.	mgm.
1. ⎫ 2.5 gm. NaOH	2.80	1.40
2. ⎭	2.80	1.40
3. ⎫ 2.5 NaOH + 0.5 gm. alloy	3.20	1.60
4. ⎭	2.90	1.45
5. ⎫ 3.5 NaOH + 0.5 gm. alloy	2.90	1.45
6. ⎭	2.90	1.45
7. ⎫ 5 gm. NaOH + 0.5 gm. alloy	3.00	1.50
8. ⎭	3.10	1.55
9. 5 gm. NaOH + 0.5 gm. alloy	3.50	1.75
10. 5 gm. NaOH + 1.0 gm. alloy	2.80	1.40
11 5.5 gm. NaOH + 0.5 gm. alloy	3.05	1.52
12. 5.5 gm. NaOH + 1.0 gm. alloy	3.00	1.50

* Solution obtained by hydrolyzing and aerating 0.2 gm. of lactalbumin.

TABLE 3

Effect of amino acids, asparagin and variations in the amount of alkali on the determination of nitrate in Devarda's method applied to plant extracts

Solution containing amino acids* 0.02 gm. asparagin (2 mgm. amide N), and 4.45 mgm. nitrate N from NaNO₃ treated as below.

TREATMENT	N/28 NaOH	N	NITRATE N	AMIDE N
	cc.	mgm.	mgm.	mgm.
1. ⎫ 2.5 gm. NaOH	6.80	3.40‡		1.95
2. ⎭	6.75	3.38		1.95
3. ⎫ 2.5 gm. NaOH + 1 gm. alloy	15.60	7.80	4.41	
4. ⎭	15.10	7.55	4.16	
5. ⎫ 3.25 gm. NaOH + 1 gm. alloy	15.40	7.70	4.31	
6. ⎭	15.50	7.70	4.36	
7. 3.25 gm. NaOH + 1 gm. alloy†	15.30	7.65	4.26	
8. Pure NaNO₃			4.45	

* Amino acids obtained by hydrolysis of 0.2 gm. of lactalbumin and subsequent removal of ammonia.

† 2.5 gm. NaOH added before reduction. 0.75 gm. NaOH added after reduction but before distillation.

‡ Mgm. N without asparagin = 1.45.

of amino acids and asparagin does not affect the difference in ammonia distilled from the controls and from the reduced solution.

Some loss was observed on boiling prior to reduction. Why this was true was not clear except that it was more difficult to keep all the liquids boiling at the same rate in the open than in a distillation. The error came principally from the incomplete volatilization of "nitrogen from sodium hydroxide" present in the controls (no. 5 and 6). The figures show that nearly half the "nitrogen from sodium hydroxide" was recovered in the distillation (table 4). A later experiment showed that better results were obtained from boiling for

TABLE 4

Effect of previous concentration of solution and boiling with NaOH in the open on the determination of nitrate in Devarda's method

4.9 mgm. nitrate N + 2.5 gm. NaOH + 0.02 gm. asparagin (2 mgm. amide N) + amino acids from arachin + 250 cc. H$_2$O treated as shown below.

	TREATMENT	N/28 NaOH	NITRATE N
		cc.	*mgm.*
1. 2.	Control for 3 and 4	7.8 7.7	
3. 4.	1 gm. alloy	17.3 17.1	4.85 4.70
5. 6.	Control for 7 and 8	1.7 1.4	
7. 8.	Same as 3 and 4 except boiled in open ½ hour before reduction	9.7 9.5	4.05 3.95
9. 10.	Same as 3 and 4 except first evaporated from 20 to 2 cc. on asbestos sheet on hot plate before reduction		2.80 2.70
11. 12.	Same as 3 and 4 except no nitrate	7.8 7.6	

1 hour. However, the previous boiling when controls are run is unnecessary and tends to decrease rather than increase the accuracy of the method. Evaporation to a low volume on a hot plate causes a loss in nitrate. A later experiment shows that the solution can be evaporated on the water bath wihout loss of nitrate. The presence of the alloy in a non-nitrate solution gives the same result as the same solution containing nitrate but not reduced by the alloy.

The effect of the aldehyde group of glucose (which is usually present in plants) on the reduction of nitrates during this determination also was studied. The results are given in table 5.

It is seen that the presence of the sugar does not affect the accuracy of the method. The loss on boiling with NaOH for 1 hour is so slight that no con-

clusions can be drawn except that apparently previous boiling does not increase the accuracy of the method. The evaporation of the neutral solution to small volume on the water bath did not apparently affect the nitrate-nitrogen results. This last experiment was made because evaporation is necessary in the determination of nitrite.

TABLE 5

Effect of glucose on the determination of nitrate in Devarda's method

Solution containing 4.9 mgm. nitrate N and 0.1 gm. arachin (hydrolyzed and aerated) + 0.01 gm. asparagin + 0.25 gm. glucose in 250 cc. H₂O treated as shown below.

	TREATMENT	N/28 NaOH	NITRATE N
		cc.	*mgm.*
1. 2.	2.5 gm. NaOH + 1 gm. alloy	13.25 13.15	4.70 4.63
3. 4.	Control for 1 and 2	3.80 3.80	
5. 6.	Same as 1 and 2 except boiled in open 1 hour before reduction	9.40 9.00	4.28 4.48
7. 8.	Control for 5 and 6	0.50 0.40	
9. 10.	Same as 1 and 2 except before reduction and addition of alkali evaporated from 25 to 3 cc. on H₂O bath	11.40 11.00	4.85 4.65
11. 12.	Control for 9 and 10	1.65 1.70	

TABLE 6

Comparison of nitron and Devarda (reduction) methods for nitrates in plants

PLANT EXTRACT NUMBER	NITRATE N IN DRY MATTER		
	Devarda	Nitron	Difference
	per cent	*per cent*	*per cent*
2	0.330	0.418	0.088
5	0.375	0.440	0.065
7	0.389	0.436	0.047
8	0.200	0.244	0.044

It is seen from the above that nitrate nitrogen in plants can be quite accurately determined by the use of Devarda's alloy. This method is not applicable in the presence of nitrites. The procedure is similar to that outlined in the third paragraph of page 341. When nitrates only are being determined it is preferable to remove the soluble protein by coagulation by heat prior to the determination. This eliminates frothing and the necessity of using paraffin.

A comparison of the nitrate content of plants as shown by the nitron method and the Devarda method is found in table 6. The former method gives consistently higher results. Apparently there are present in plants compounds which cause an appreciable error by this method.

As already mentioned the Schloesing method, variously modified, for nitrates has been used by most workers in their studies on the nitrate content of plants. This method is more complicated and requires more manipulation than the Devarda method. However, on account of its wide use for the determination of nitrates in plant tissue comparative studies were made of the methods mentioned. The Devarda method modified as already described, was used in comparison with the modified Schloesing method as described by Treadwell and Hall (12, p. 456) with the further modifications suggested by Koninck (6) and Koch (13).

TABLE 7

Comparison of Schloesing's and Devarda's methods for nitrate in plant tissue

	DEVARDA'S	SCHLOESING'S
Pure NaNO₃ (mgm. N)..................................	13.95	13.81
	2.79	2.71
Nodule extract...	None	None
Plant extract (Nitric N, per cent of dry matter)............	0.260	0.245
	0.200	0.178
	0.288	0.276
Plant juice (mgm. in 100 cc.)...........................	30.0	23.7
	90.0	86.1

The first modification was a mechanical one whereby a mercury seal was substituted for a pinchcock for the tube leading to the gas burette. The Koch modification consisted in measuring the evolved gas absorbed by alkaline potassium permanganate instead of the total gas evolved.

Comparative determinations by this method and by the Devarda method are shown in table 7. In every case but one the results agree almost as closely as duplicate determinations by the same method. In the Schloesing method a small amount of gas was invariably found after absorption.

II. Nitrites and nitrates in the presence of one another

No attention was given in the above studies to the question of nitrites. Devarda's method would include both nitrites and nitrates, while with the nitron method according to Treadwell and Hall (12, p. 451) nitrites cause high but not quantitatively high results.

Zeller's method for nitrates and nitrites has already been mentioned. As stated, he claimed this method to be accurate for soils and plant decoctions. He does not describe the preparation of these decoctions but gives figures which indicate very accurate results. When small amounts of nitrite are present a large percentage of error will probably be found in plant tissue, especially for seedlings, since amino acids are always present which are much more reactive with nitrous acid than with ammonia. Alpha amino nitrogen reacts quantitatively with nitrous acid on shaking in acid solution for five minutes at room temperature, whereby from 1 to 1½ hours are required for the complete reaction with ammonia under the same conditions (10). In 17-day-old soybean plants 0.65 per cent α amino nitrogen was found. It is possible that a considerable amount of nitrite could be present in such cases with no loss whatever of ammonia by Zeller's method.

This error, however, could probably be avoided by the following procedure. Treat the unknown solution with ammonium chloride solution, make up to a definite volume and divide into two exactly equal portions or take similar aliquots. Determine the total nitrogen in one part immediately, and in the other after evaporating to a low volume on the water bath. Half the difference in nitrogen = nitrous nitrogen.

A simpler and shorter method which might be used for determining both nitrite and nitrate is as follows. With one aliquot of the unknown solution determine nitrate plus nitrite by Devarda's method as already outlined. Treat another aliquot with an excess of some amino compound which reacts readily with nitrous acid and which does not lose ammonia on boiling with sodium hydroxide. Heat on the water bath for a definite time. The nitrous acid reacts with the amino acid forming elemental nitrogen. Dilute to 250 cc. and determine nitrate by Devarda's method. The difference between the first and the second determination gives the nitrite nitrogen.

This method was tried and was found to give good results in the presence of nitrate and a cold-water extract of 14-day-old etiolated soybean seedlings. The amino compound fitting the above description which happened to be available was aspartic acid and this accordingly was used.

Merck's sodium nitrite, made up to equal approximately 6 mgm. of nitrite nitrogen, was used. This was found by reduction with Devarda's alloy to contain 5.57 mgm. of reducible nitrogen of which 5.2 mgm. was lost on heating on the water bath for 1 hour in initial solution of 20 cc. (evaporated to 5 cc.) with 0.15 gm. aspartic acid. No more nitrogen was lost on heating with 0.3 gm. aspartic acid to a lower volume. The plant extract was prepared by shaking vigorously by hand 8 gm. of finely ground seedlings with 100 cc. of cold water for 5 minutes, allowing to stand for about 20 minutes and again shaking for 5 minutes. This extract was then squeezed through a cloth. No attempt was made to remove soluble protein by heat or acid. The results are given in table 8. It is seen from the table that fairly accurate results are obtained. It is noted from the last pair of figures that nearly half the nitrite is lost through

the evaporation of 5.2 mgm. of nitrite nitrogen with 0.5 gm. of tissue extract without the addition of aspartic acid. This shows the fallacy of attempting to use the procedure proposed by Zeller for nitrite in plant tissue.

One per cent aqueous sodium alizarin sulfonate was used as an indicator in all this work and was found to be very satisfactory in titrating small amounts of ammonia. With the amounts employed a distinct change in color was noted at the end point with one drop of 0.0357 N alkali.

The question of possible losses of nitrites during extraction of the tissue, and the means by which such losses (if they exist) may be minimized or eliminated is still open for investigation.

TABLE 8

Results of test on method for nitrite and nitrate

5.2 mgm. Nitrite N + 3.8 mgm. Nitrate N + 0.5 gm. seedlings extracted + 0.15 gm. aspartic acid treated as indicated.

TREATMENT	TOTAL N/28 NaOH	NITRATE + NITRITE	NITRATE N	NITRITE N
	cc.	mgm.	mgm.	mgm.
1.⎫ Heated on water for 1 hour, reduced and dis-	21.4	3.5	3.5	
2.⎭ tilled	21.7	3.8	3.8	
3.⎫ Control, no alloy	18.1			
4.⎭	17.7			
5.⎫ Reduced and distilled, no previous heat	30.9	13.5		4.85
6.⎭	31.0	13.6		4.90
7. Control for 5 and 6	17.4			
8.⎫ Same as 1 and 2 except no aspartic acid	26.7	9.3		2.75
9.⎭ added	26.9	9.5		2.85
HNO₂ by reduction........................			3.8	
HNO₂ by reduction........................				5.17

Procedure. Dilute two equal portions of a cold-water extract of plant tissue to 250 cc. in a Kjeldahl flask. Add a small piece of paraffin and 2.5 gm. of sodium hydroxide in concentrated solution. To one solution add 1 gm. of Devarda's alloy and use the other as a control. Attach to a distilling apparatus at once. Heat under a low flame for 1 hour or until action has ceased and then distill over exactly 150 cc. Care should be taken that the determination and the control be distilled at the same rate. Titrate, using 0.0357 N alkali. The difference gives the nitrate plus nitrite nitrogen. The flask is attached to a distilling head containing two way bent tubes.

Treat a similar portion of cold water extract in a volume of about 25 cc. with about 0.15 gm. of aspartic acid or more depending upon the amount of nitrite present. The mixture is heated on the water bath for an hour. It is then divided into two equal portions, reduced and distilled according to the

Devarda method as given above. The difference between the first and second distillations represents the nitrite nitrogen.

SUMMARY

1. The Caron colorimetric method for nitrates in urine is not applicable to the determination of nitrates in plant tissue.

2. The determination of nitrates in plants by finding the difference between the Kjeldahl-Gunning-Arnold method and the Kjeldahl method modified to include nitrates is unsatisfactory.

3. The "nitron" method gave slightly high results with the tissue studied. In view of the substances occurring in plants which may cause error in this method it is not dependable for the determination of nitrates in plant tissue.

4. Both the Devarda and Schloesing methods with proper modifications may be applied in the determination of nitrates in plants with fair accuracy.

5. The method proposed by Zeller for the determination of nitrites and nitrates in the presence of one another is not applicable to plant tissue.

6. A procedure is suggested which gives satisfactory results for the determination of nitrites and nitrates in plant extract.

REFERENCES

(1) ALLEN, E. R. 1915 The determination of nitric nitrogen in soils. *In* Jour. Indus. Engin. Chem., v. 7, p. 521.

(2) Association of Official Agricultural Chemists 1908 Official and provisional methods of analysis. U. S. Dept. Agr. Bur. Chem. Bul. 107.

(3) BUSCH, M. 1905 Gravimetrische Bestimmung der Saltpetersaure. *In* Ber. Deut. Chem. Gesell., Bd. 38, p. 861.

(4) CARON, H. 1912 The determination of nitrates in urine. *In* Jour. Répert. Pharm. et Chim. Med., ser. 3, v. 23, p. 385. *Abs. in* Chem. Abs., v. 6, p. 2246.

(5) DAVISSON, B. S. 1918 Ammonia and nitric nitrogen determinations in soil extracts and physiological solutions. *In* Jour. Indus. Engin. Chem., v. 10, p. 600.

(6) KONINCK, L. S. DE 1894 Eine neue Ausfuhrungsweise der Schlosing'schen. Salt-petersaurebestimmungsmethode. *In* Ztschr. Anal. Chem., Bd. 33, p. 200.

(7) KROG, K., AND SEBELIEN, J. 1911 Ueber die Quantitative Bestimmung von Salt-petersaure in Pflanzenstoffen. *In* Chem. Ztg., Bd. 35, p. 145.

(8) NEDOKVOCHAYEV, N. 1904–05 Conditions of accumulation and reduction of nitrates in plants. *In* Izv. Moscov. Selsk. Khoz. Inst. (Ann. Inst. Agron. Moscou), v. 10, no. 1, p. 257–347; *Abs. in* Exp. Sta. Rec., v. 16, p. 1053.

(9) OSBORNE, T. B. 1912 The Vegetable Proteins, p. 63. Longmans, Green & Co., New York.

(10) PLIMMER, R. H. A. 1912 Chemical constitution of the proteins, pt. 1, p. 72. Long-mans, Green & Co., New York.

(11) SCHULZE, E. 1897 Ueber das Vorkommen von Nitrates im Keimpflanzen. *In* Ztschr. Physiol. Chem., Bd. 22, p. 82.

(12) TREADWELL, F. P., AND HALL, W. T. 1919 Analytical Chemistry, v. 2, 5th ed. John Wiley and Sons, New York.

(13) WOO, M. L. 1919 Chemical constituents of *Amaranthus retroflexus*. *In* Bot. Gaz., v. 68, p. 313–344.

(14) ZELLER, T. 1909 Eine einfache Methode zur Bestimmung des Nitrat-und-Nitrit-stickstoffs in Gemischen und in Gegenwart organischer Substanzen. *In* Landw. Vers. Stat., Bd. 70, p. 145.

THE RELATION OF NITRATES TO NODULE PRODUCTION[1]

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INTRODUCTION

When inoculated soybeans are grown in pure quartz sand, to which has been added an abundance of the essential plant-food elements except nitrogen, the bacteria will assimilate sufficient nitrogen to meet the normal demands of the plant. On the other hand, the soybeans will thrive equally as well without bacteria, provided sufficient combined nitrogen is supplied in available form; for example, nitrates. For the farmer it is important to know what proportion of the nitrogen of the legumes is obtained from soil and what portion is obtained from the air by means of the bacteria. The relation that exists under ordinary field conditions has been the subject of considerable study and discussion for some time, and any contribution to our knowledge of the factors governing nitrogen fixation will aid in its ultimate solution.

Many investigators have shown that certain salts inhibit, and in sufficient quantities entirely prevent, nodule formation on legume plants. Why this is true is not known and although several explanations have been offered none of these is based upon satisfactory experimental data. The purpose of this paper is to attempt to offer an explanation of the deleterious effects of large amounts of nitrates on nodule formation. While an exhaustive review of the literature is unnecessary some of the important papers will be discussed.

Effect of nitrates on legume bacteria

Hiltner (6) found that nitrates inhibited nodule formation and that the inhibiting effect of a given concentration of nitrates was much greater in solution than in soil. Prucha (15) reported that nitrates inhibited nodule formation in the Canada field pea. Wilson (19) studied the effect of various salts on nodule formation. He showed that nitrates and sulfates retarded nodule formation, while chlorides and phosphates did not. He also found

that the inhibition was local in character and when sufficient nitrate was present in soil to prevent nodule formation, the vitality of the legume organism present *in the soil* was not weakened.   Fred and Graul (3) found that nitrates and ammonium salts markedly inhibited the development of nodules on vetch, alfalfa and soybeans.   Mazé (10) believed that the retarding effect of nitrates was due to two causes; first, that the bacteria found sufficient nutrition outside the plant and did not enter it; and, second, that nitrates reacted with the sugar in plants and thus prevented the bacteria from obtaining sufficient sugar for their development.   It has also been suggested that the immunity of the plant is strengthened by the presence of nitrogen in the form of nitrates so that the bacteria do not enter it.   However, the data presented in support of any of these theories are far from convincing.   Furthermore, none of them explain why certain non-nitrogenous salts, e.g. sulfates, also prevent nodule formation.   It is known, that the nodules diminish in size and number with the amount of nitrate until, with sufficient nitrate present, no nodules are found.   If the first theory of Mazé is correct there should be no diminution in size; instead there should be either well developed nodules or none at all.   It is noted that nodules develop on plants before the reserve food of the seed is exhausted.   This is out of harmony with the last-named theory.   The second theory of Mazé will be considered at a later time.

Laurent (9) stated that sodium or potassium nitrate in pea or lupine decoctions in a ratio of 1 to 500 or 1 to 1000 prevented growth of legume bacteria. He stated that the nitrate alone, or the decoction alone, has no such effect. He, therefore, believed that the nitrate reacts with some compound in the plant and forms thereby a substance toxic to bacteria.

Wilson (19) has shown that when soybean bacteria are placed in a soil containing sufficient nitrate to prevent plants growing therein from forming nodules, the vitality of these organisms, as shown by their infecting power, was apparently not weakened.   Hills (5) confirmed Wilson's results by using alfalfa bacteria and mannite agar slopes instead of soil.   He employed a nitrate concentration up to 100 mgm. of nitrate nitrogen per 100 gm. of media.   While these data show that the organism is not destroyed by relatively large amounts of nitrate, they do not prove that growth and reproduction are not hindered to a marked degree.   In fact, Hills (5) showed in his studies on the effect of nitrates upon growth and reproduction of *Rhizobium leguminosarum* in soil, that while small amounts of nitrate greatly stimulated reproduction, higher concentrations produced a toxic effect, and reproduction was diminished to less than 1 per cent of the normal in untreated soil.

A fact that must be considered in connection with such studies is that the bacteria of the nodules live within, and obtain their nutriment from the plant itself, and not from the soil or its solution.   As regards mineral nutrients absorbed from the soil the concentration in plant sap and in soil solution are far from equal.   McCool and Millar (11) showed by osmotic-pressure determinations that roughly the concentration of plant sap increased with the

concentration of the soil solution, but the increase was not proportional. Hoagland (7) recently showed by conductivity measurements, that while the concentration of the sap varied considerably with that of the soil, the concentration of the former was 5 to 50 times greater than that of the latter. The data recorded herein (most of which were obtained before Hoagland's paper was published) are in harmony with these results.

Since Fred and Davenport (4) have shown that the different strains of *Rhizobiun leguminosarum* are sensitive to the reaction, the possibility is suggested that the toxic action of nitrates may be due to their causing a change in the hydrogen-ion concentration of the sap. While apparently no studies have been made upon the effect of nitrates on reaction, the work of Hoagland (7), Truog and Meacham (18), and Clevenger (1), show that the reaction of the plant sap is only slightly affected by the composition or reaction of the soil solution, since the reaction of the former is governed apparently by a definite buffer system.

Nitrates in plants

Schulze (16) found no nitrate nitrogen in plants grown in soil entirely free of nitrates, but nitrates were present in considerable amounts when an external supply was provided. Nedokvochayev (12) found that the nitrate content of plants increased with the nitrate content of soil although not proportionally. Woo (20) found large amounts of nitrate in *Amaranthus retroflexus* (pigweed). The amount varied at different stages of growth and in different parts of the plant.

EXPERIMENTAL

From the above discussion it is seen that while the composition of the soil solution may modify to some extent, it does not govern entirely, the composition of the plant sap. Therefore, with the object in view of trying to explain why nitrates inhibit nodule formation, comparative studies of the sap from plants treated differently were undertaken. Determinations were made of the different forms of nitrogen (nitrate, amino, amide and basic), of sugar, and of the hydrogen-ion concentration.

Since the data obtained for the various non-nitrate forms of nitrogen threw no light on this problem, they are not considered in this paper.

In order to study the effect of nitrates on nodule formation, Ito San soybeans were grown in 2-gallon jars containing 11 kilos of air-dry sand. The sand was held at 14 per cent moisture throughout the experiment. One week after planting, half of the jars were inoculated with a pure culture of *Rhizobium leguminosarum*, while the other half were treated with 1 gm. of calcium nitrate per jar each week. Every week the following nutrient solution was added to all jars: 10 cc. of 1 per cent disodium phosphate, 10 cc. of 1 per cent magnesium sulfate, 10 cc. of 2 per cent potassium sulfate and 1 cc. of 0.1 per cent ferric chloride. After 14 days the inoculated plants contained an abun-

dance of nodules, while the uninoculated plants remained entirely free from nodules. The plants were harvested when 24, 38 and 44 days old. The plants were in full bloom at the second harvest and at the third harvest young pods were just beginning to appear. The third harvest was made earlier than was expected on account of the threatened ravages of parasites. All plants were in good condition at the time of harvest. The data of the uninoculated plants are given in table 1.

Methods

The modified Devarda and modified Schloesing methods for nitrates were used. These methods are discussed in detail in part I of this paper.

Inoculated plants gave faint qualitative tests for nitrates but the amount was not measurable with the tissue available. The plants grown in sand receiving nitrates contained a large percentage of nitrate nitrogen—the fresh green plants contained from 7 to 40 times the percentage of nitrate nitrogen that had been added to the soil (table 1).

TABLE 1

Nitrate nitrogen in different parts of soybean plants; uninoculated

Greenhouse sand cultures

PART OF PLANT	AGE OF PLANT	NITRATE N ADDED TO 100 GM. OF SOIL	NITRATE N IN AIR-DRY PLANTS	NITRATE N IN 100 GM. OF FRESH GREEN PLANTS
	days	*mgm.*	*per cent*	*mgm.*
	24	4.5	0.875	175
	24	4.5	0.815	163
Tops............	38	9.0	0.359	72
	38	9.0	0.458	92
	44	10.5	0.440	88
	44	10.5	0.375	75
Roots............	44	10.5	0.428	86
	44	10.5	0.472	95

Occurrence of nitrates in plants grown in the field

Mammoth yellow soybeans inoculated with a pure culture of bacteria were grown on a farm near Madison. The soil was quite fertile and had received liberal treatments of farm manure for years, as well as occasional applications of limestone and floats. Perhaps on account of the richness of the soil the nodules were not very large compared with those grown in sand, although they were found in considerable numbers in all plants except in local areas throughout the field. On these areas, usually a few feet in diameter, the plants were found free of nodules, but equally vigorous and somewhat richer in nitrogen. These areas may have represented former manure piles, although no definite information could be obtained on this point. Uninoculated and inoculated plants were collected at the flowering stage and the leaves, stalks and roots

were analyzed (table 2).   The leaves and stalks show very decided differences in nitrate content.   The difference in the nitrate content of the roots was not so marked, but in both cases the concentration was relatively high.   The data for tops and stalks show that at the earlier stages of growth the variation was possibly much greater.   As further evidence that such was the case, the work of Stewart (17) shows that the nitrate content of soil is richer in the early part of the growing season than later, and McCool and Millar (11) show that roots are more sensitive to change in composition of nutrient solution than are the tops.   The very low nitrate content of the nodules compared with the roots is interesting.   The use of nitrate by the bacteria probably accounts for this decrease.

TABLE 2

Nitrates in inoculated soybean plants, with and without nodules, grown in the same field

Samples taken from a field near Madison

PART OF PLANT	STAGE OF GROWTH	OCCURRENCE OF NODULES	NITRATE N IN AIR-. DRY PLANTS	NITRATE N IN 100 GM. OF FRESH GREEN PLANTS
			per cent	*mgm.*
Leaves...........	Flowering	Present	0.063	12.6
	Flowering	Present	0.075	15.0
	Flowering	Absent	0.125	25.0
	Flowering	Absent	0.123	24.0
Stalks............	Flowering	Present	0.075	15.0
	Flowering	Present	0.056	11.2
	Flowering	Absent	0.175	35.0
	Flowering	Absent	0.175	35.0
Roots.............	Flowering	Present	0.088	17.6
	Flowering	Present	0.085	17.0
	Flowering	Absent	0.100	20.0
	Flowering	Absent	0.095	19.0
Nodules...........	Flowering		0.005	1.0

Occurrence of nitrates and sugar in plants grown in varying concentrations of nitrates

In order to determine the concentration of nitrate in soil and plant juice at which nodules failed to grow, plants were grown in sand containing different concentrations of nitrate.   Two-gallon jars containing 11 kilos of air-dried sand and 10 gm. of calcium carbonate each were used and enough water added to bring the content up to 15 per cent.   The desired quantity of sodium nitrate was added in the water.   For each concentration of nitrate 8 jars were planted with Medium Early Green soybeans.   The plants were removed from 4 jars for each duplicate determination.   All jars were inoculated when the plants were 12 days old and again a week later.   The nutrient solution was added weekly,

beginning 14 days after the soybeans were planted. The same kind and amounts of non-nitrogenous nutrients that were used in the former greenhouse experiment also were added in these tests.

Nodules were observed first in the control plants when 22 days old, or 10 days after the first inoculation. The plants were harvested when 31 days old. Immediately after harvesting the roots were washed free from sand in running water and then shaken as free as possible from water. The roots were then cut off from the tops and chopped into fine pieces. This finely cut tissue was then thoroughly macerated in an agate mortar and the juice squeezed through canvas into a test tube. The test tube was immediately corked and placed in a freezing mixture of ice and salt. About 15 minutes elapsed between the

TABLE 3

Effect of nitrates on reducing sugar and nitrate content of plant sap (afternoon harvest)

Plants grown in sand

CONDITION OF PLANTS AT HARVEST	NUMBER AND SIZE OF NODULES	NITRATE N ADDED TO 100 GM. OF SAND	NITRATE N IN 10 CC. OF PLANT JUICE		REDUCING SUGAR IN PLANT JUICE*
			Tops	Roots	Roots
		mgm.	mgm.	mgm.	mgm.
Thrifty............................	Large; abundant	0	36.0	4.0	342
	Small, few	2.5	93.7	47.0	421
Thrifty, smaller than above..........	None	5.0	93.7	56.1	187
	None	10.0	100.0	72.2	168
No germination.....................		20.0			

* Calculated as mgm. of dextrose per 100 cc. of juice.

harvest and placing the extracted juice of tops and roots in the freezing mixture. The juice was kept cold until the analyses were made. The analyses were completed within 48 hours after harvest. The results are given in table 3.

The data are in conformity with those made on the dry matter. The nitrate content of the juice increases with the nitrate content of the soil, although the increase is not nearly so great proportionally.

The reducing sugar content decreases with the increase in nitrate, although a considerable amount is present even with the highest concentration of nitrate.

These plants were harvested in the afternoon when the sugar content probably approached the maximum. In order to determine the sugar and nitrate content of the root sap in the early morning another set of plants was grown.

For this second experiment new sand was obtained for the controls while the same sand which originally contained 5, 10 and 20 mgm. of nitrate nitrogen, respectively, used in the previous experiment, was again used.

The plants were harvested when 26 days old and had attained approximately the same stage of growth as those in the previous experiment. The control plants were somewhat larger than the plants grown in the lowest concentration of nitrate. Ten milligrams of nitrate depressed growth to a greater extent while the seed again failed to germinate in the sand containing 20 mgm. of nitrate nitrogen per 100 gm. The harvests and extractions were made from 6 to 7 a.m.

The control plants contained large nodules while the plants from the first concentration contained very small nodules, barely visible to the naked eye. The plants grown in the higher concentration were entirely free from them. The results are given in table 4.

The reducing sugar content was lower and the nitrate content higher in plants collected in the morning than in those collected in the afternoon. The plants not being grown at the same time, of course, are not exactly comparable.

TABLE 4

Effect of nitrates in sand on reducing sugar and nitrate content of plant sap

Early morning harvest

HEIGHT OF PLANTS	GER 'ON	NITRATE N ADDED TO 100 GM. OF SAND	IN 100 CC. OF ROOT JUICE	
			Nitrate N	Reducing sugar
inches		*mgm.*	*mgm.*	*mgm.*
7½	Good	0	17.5	220.0
6	Good	5	87.5	107.4
5	Less than 50 per cent	10	120.0	75.8
0	None	20		

The accumulation of nitrates in darkness

The figures given in tables 3 and 4 indicate that nitrates accumulate in plants during the night. It is, therefore, interesting to know to what extent they may accumulate during longer periods of inhibited photosynthesis. The following studies, therefore, were made.

Jars containing 37-day-old plants grown in sand to which 10.5 mgm. of nitrate nitrogen from calcium nitrate had been added were removed to a dark cellar. Three days later another lot of the same plants was placed in the cellar. Jars were kept also in the light for controls.

All plants were harvested when 44 days old and analyzed for nitrate. The results are given in table 5.

It is seen that there was a decrease of nitrate in the tops but an increase in the roots. This may possibly be explained on the basis of decreased transpiration and the consequent decreased upward movement in the cool, damp cellar. The residual sugar in the leaves used up the nitrates faster than it was moved upward, although not as fast as it was absorbed by the roots. As further evidence that such an explanation may be the correct one, it was

found that when 38-day-old plants were placed in a warm, dry, dark closet for 3 days the percentage of nitrate in the tops was greatly increased.

From the data presented it appears that in the other experiments the nitrate content of roots when harvested probably was as low as or lower than at any other time of the day, with the exception of the results reported in table 4.

TABLE 5

Effect of darkness on the accumulation on nitrates in plants

Sand cultures

AGE OF PLANTS IN LIGHT	TIME KEPT IN DARKNESS	NITRATE N ADDED PER 100 GM. OF SAND	NITRATE CONTENT IN 100 GM. OF SAP	
			Tops	Roots
days	*days*	*mgm.*	*mgm.*	*mgm.*
44	0	10.5	110	118
44	0	10.5	83	107
40	4	10.5	77	140
37	7	10.5	61	145

Nitrate content of roots from the same plant when divided parts are placed in nitrate and non-nitrate solutions

Wilson (19) reported that when the roots of a soybean plant were divided and one portion placed in an inoculated solution containing no nitrate and the other portion in an inoculated solution containing nitrate, the roots in the non-nitrate solution developed nodules while those in the nitrate solution developed none. He concludes that the inhibiting factor is local in character. If, as the data reported thus far indicate, there is a connection between the nitrate content of the plants, and the failure of the plants to form nodules, the nitrate content of two root portions should differ. Accordingly, experiments were made to determine whether or not such a variation existed.

The amount of nitrate in the divided root portions was determined as follows. In two ordinary glass tumblers was placed 250 cc. of a nutrient solution without nitrogen. To one of these tumblers was added sufficient calcium nitrate to give a 0.05 per cent concentration of the salt (20 mgm. of nitrate nitrogen). Roots from 6 inoculated soybean plants (40 days old) were divided as evenly as possible and one portion was placed in each tumbler. The water lost was renewed daily. Roots from 24 plants were harvested after 3 days for duplicate determinations. The roots from each portion were cut off and thoroughly washed, and after shaking as free as possible from adhering water the roots were blotted between filter paper. They were then cut up fine, macerated in a mortar and the juice squeezed through canvas. The nitrate determinations were completed within 3 hours after harvesting.

To another set of plants a second addition of 20 mgm. of nitrate nitrogen as calcium nitrate was added to the nutrient solution after the fifth day.

These plants were allowed to remain in the solution for a total of 8 days. The results with the two sets of plants are given in table 6.

The non-nitrate solution gave a negative qualitative test for nitrate at the end of the 8-day period.

These results show a decided difference in the nitrate content of the roots in the two portions. This is in harmony with the results of Nedokvochayev (12) and of Kraus and Kraybill (8) who have shown that different parts of the same plant organs may differ in nitrate content.

TABLE 6

Nitrates in different portions of roots of the same plants (40 days old) when different portions are placed in nutrient solutions with and without nitrates

DAYS IN SOLUTION	NITRATE QUALITATIVE	NITRATE	
		In 100 cc. of nutrient solution	In 100 cc. of juice.
		mgm.	mgm.
3	Present	0	Trace†
3	Present	0	Trace†
3	Present	8.0	8.46
3	Present	8.0	8.46
8	Present	0	1.20
8	Present	0	0.90
8	Present	16.0*	10.16
8	Present	16.0*	Lost

* 8 mgm. added at beginning; 8 mgm. added after 5 days.
† Less than 1 mgm. per 100 cc. of juice.

The effect of nitrates upon the reaction of the plant juice

It has been noted earlier in this paper that several investigators have shown that the reaction of the plant sap is governed by a buffer system, and that this reaction is to a considerable degree independent of the composition and reaction of the soil solution. However, since the specific effect of nitrates on reaction had not been studied, the determinations of hydrogen-ion concentration of the juice from the same set of plants reported in table 4 were made. Plants 26 days old were harvested in the afternoon, and the determination made immediately. It was found that untreated plants had a hydrogen-ion concentration, expressed as pH, of 5.82, plants receiving 5 mgm. of nitrate nitrogen had a pH of 6.14 and plants receiving 10 mgm. a pH of 6.4.[2]

These figures show that sodium nitrate decreases the acidity (H-ion concentration) to a slight extent, but the pH in the nitrate-containing plants is even more favorable for the growth of *Rhizobium leguminosarum* than the reaction of the sap of plants containing no nitrate. Apparently, the inhibiting effect of nitrates on nodule production is not a question of reaction.

[2] These determinations were made by O. C. Bryan.

It was pointed out in the introductory part of this paper that two theories explaining why nitrates inhibit nodule production are not tenable in the light of observed facts. Experiments were made to determine the validity of the other theory, namely, the failure of the bacteria to thrive on account of the lack of sugar, since the sugar was used up in reacting with the nitrate. The data obtained showed that while the sugar content was less in the plants containing nitrate, the soluble reducing sugar did not entirely disappear from the nitrate-containing plants even in the early morning after the nocturnal period of the arrested photosynthesis.

From the studies reported herein it is seen that when soybeans are grown in soil or sand containing nitrate there is a marked accumulation of nitrates in the roots and tops of the plants and that normally, the concentration of nitrate is much greater in the plant than in the soil, or even in the soil solution. It was also observed that nitrates in the plants increased to some extent with the increase of the nitrates in the soil. Furthermore, when plants are grown in a sand very poor in nitrate, comparatively small amounts of nitrate are found in the plant.

Hills' (5) results on the effect of nitrates on the growth and reproduction of *Rhizobium leguminosarum* are given in table 7. It is noted from these results that the amounts of nitrates (2.5, 5.0 and 10 mgm. of nitrate nitrogen per 100 gm. of soil) added to sand in the greenhouse experiments reported in this paper, actually stimulated growth and reproduction, but that *the concentration of nitrate similar to that found in the plant when nodule production was inhibited, was sufficient to bring growth and reproducion of the bacteria in the soil to a virtual standstill.* The decrease in growth due to the increase in nitrate is gradual, just as in the plants there is a gradual decrease in the size of the nodules with an increase in the amount of nitrate. Likewise, the analyses of plants containing small nodules grown in a fertile field showed enough nitrates, according to the table, to have some inhibiting effect upon the growth of *Rhizobium leguminosarum*. Inoculated plants entirely free from nodules, from the same field, contained decidedly more nitrate.

It was shown that when different portions of roots from the same plant are grown in nitrate and non-nitrate solutions the nitrate content of the portion grown in nitrate was 10 times greater than the portion grown in the non-nitrate solution.

Whenever an inhibiting effect on nodule production occurred in these studies sufficient nitrate was found *in the plant* to check to a considerable degree the reproduction of *Rhizobium leguminosarum* in soil as shown in table 8. The amount of nitrate present in the plant was several times greater than the nitrate content of the soil in which the plant was grown; also there was no definite relationship between the nitrate content of the soil and that of the plant. On account of relatively rapid diffusion in the plant sap the nitrate consumed

by bacteria in the plant roots would probably be more quickly replaced than would be the case in either soil or agar. Therefore, aside from the buffer actions of the soil particles on substances toxic to organisms, a given concentration of nitrate may be more effective in inhibiting the growth of bacteria in the sap than in the soil or agar. It is obviously impossible to determine the exact effect of a specific concentration of nitrate upon legume bacteria in the

TABLE 7

Influence of calcium nitrate on Rhizobium leguminosarum in sterilized soil
Results obtained by Hills (5)

CULTURE NUMBER	TREATMENT* (NITRATE N IN 100 GM. OF DRY SOIL)	NUMBER OF ORGANISMS IN 1 GM. OF DRY SOIL				
		At the beginning	After 1 week	Relative	After 2 weeks	Relative
	mgm.			*per cent*		*per cent*
1		10,000	960,000	100	4,675,000	100
2		10,000	850,000		4,590,000	
3	2.3	10,000	3,650,000	419	6,000,000	124
4		10,000	3,940,000		5,450,000	
5	6.0	10,000	5,500,000	674	10,650,000	274
6		10,000	6,700,000 °		14,700,000	
7	11.5	10,000	4,000,000	414	9,350,000	195
8		10.000	3,500,000		8,670,000	
9	23.0	10,000	1,200,000	180	1,500,000	35
10		10,000	2,050,000		1,750,000	
11	35.0	10,000	865,000	106	765,000	17
12		10,000	1,050,000		800,000	
13	46.0	10,000	375,000	35	350,000	7
14		10,000	260,000		300,000	
15	69.0	10,000	35,000	4.5	25,000	0.7
16		10,000	47,000		40,000	

* Hills reported the soil treatment in terms of nitrate ($+NO_3$). These figures have been converted to the nitrogen (N) equivalent in order to harmonize with the other data.

living plant root, and while, of course, the effect of a given concentration in soil cannot be exactly comparable to the effect of the same concentration of nitrate in the plants, the data reported herein, interpreted in the light of data secured by others, offer striking evidence that the inhibiting action of nitrate upon nodule formation is at least in part due to the antiseptic action of the nitrate of the root sap upon *Rhizobium leguminosarum*. That this action is probably not due to any change in osmotic pressure is seen from Wilson's (19) results, namely, that nitrates and sulfates inhibit nodule production but phos-

phates and chlorides have an opposite effect. This would appear to indicate that the inhibiting action is of a specific nature. Also data are reported above which prove that the effect is not due to a change in reaction.

Kraus and Kraybill (8) have shown that a high nitrate content in plants accompanies a low sugar content and vice-versa. Data reported herein confirmed their results. Consequently, any evidence that nitrates *in the plant* exert a detrimental effect upon legume bacteria also may be considered as evidence that a decreased sugar supply is responsible. On the other hand, the work of Hills, previously discussed, shows that the deleterious effect of an excess of nitrates on legume bacteria takes place in soil where photosynthetic processes are not involved. However, before any definite conclusion can be drawn in this regard, it will be desirable to determine whether or not the addition of sugar to the soil counteracts the depressing action of nitrate. Likewise, although sulfates and nitrates independently exert an inhibiting effect on nodule production, it may be contended that the effect of sulfates could be due to the possible stimulation of the nitrogen metabolism of the plants and the resultant increased consumption of sugar. Further studies should also be made regarding this point.

Furthermore, it would be interesting to know the relative absorption and accumulation of the various salts by legume plants. It may be noted in this connection that Peterson (13) found a large accumulation of sulfates in plants grown in soil to which sulfates had been added. The behavior of sulfates in that respect is thus seen to be similar to that of nitrates. The effect of high concentrations of sulfates upon the growth and reproduction of legume bacteria in soil or in solution has not been studied. Pitz (14) found that 1 per cent calcium sulfate had little effect upon the total number of organisms in the soil. Fred and Hart (4) studied the effect of different sulfates upon carbon-dioxide production in soil. They found in general that small amounts produced a marked stimulation in carbon-dioxide production, and that an optimum concentration was reached, after which further sulfate additions produced a decline in the carbon-dioxide output.

Another interesting study that this work suggests is the antiseptic action of absorbed radicals on phytopathogenic organisms. The statement is often made that fertilizers give the young plant more vigor and render it more resistant to disease. To determine whether this increased immunity is at least partially due to the accumulation of certain salts absorbed by the plant from the fertilizers would make a very interesting and important subject of investigation.

SUMMARY

When soybeans are grown in soil or in sand containing nitrates there is a marked accumulation of nitrates in the plant.

The concentration of nitrate in the plant sap is much greater than in the soil or even in the soil solution.

There is an increase in the nitrate content of the roots during periods of arrested photosynthesis.

The nitrate content of sap increases to some extent with the increase of nitrate in sand, although the increase is not proportional.

Nitrates retard, and in sufficient quantities entirely prevent, nodule formation.

Nitrates have little effect upon the hydrogen-ion concentration of the plant juice.

The concentration of nitrate present in plant sap when the plants fail to produce nodules is sufficient practically to prevent growth and reproduction of soybean bacteria in soil. On the other hand, the concentration of the nitrate in the soil in which the plants were grown is so low that growth and reproduction *in soil* are stimulated.

While different buffer actions and rates of diffusion make exact comparisons between the effect of different concentrations in soil and in sap impossible, the data presented offer strong evidence that the reason for failure of nodule production in the presence of nitrates is due at least in part to the effect of the high concentration of nitrate in the sap upon the growth and reproduction of *Rhizobium leguminosarum.*

This theory is not out of harmony with Wilson's findings that the inhibiting factor was local in character.

The amount of reducing sugar in plants decreases with the increase in nitrate, but sugar was present with the highest content of nitrate used even in the early morning. Further study is needed before definite conclusions can be reached as to what extent the failure of nodule production in the presence of nitrates is due to a diminished sugar supply.

Some evidence indicates that the deleterious effect of the high nitrate concentration upon *Rhizobium leguminosarum* is at least partially of a specific nature.

REFERENCES

(1) CLEVENGER, C. B. 1919 Hydrogen-ion concentration of plant juices. II. *In* Soil Sci., v. 8, p. 227–242.

(2) FRED, E. B., AND DAVENPORT, AUDREY 1918 Influence of reaction of nitrogen assimilating bacteria. *In* Jour. Agr. Res., v. 14, p. 317.

(3) FRED, E. B., AND GRAUL, E. J. 1916 The effect of soluble nitrogenous salts on nodule formation. *In* Jour. Amer. Soc. Agron., v. 8, no. 5, p. 316–328.

(4) FRED, E. B., AND HART, E. B. 1915 The comparative effect of phosphates and sulfates on soil bacteria. Wis. Agr. Exp. Sta. Res. Bul. 35.

(5) HILLS, J. L. 1918 Influence of nitrates on nitrogen assimilating bacteria. *In* Jour. Agr. Res., v. 12, no. 4, p. 183–230.

(6) HILTNER, L. 1900 Ueber die Ursachen, Welche die Grosse, Zahl, Stellung und Wirkung der Wurzelknollchen der Leguminosen Bedingen. *In* Arb. k. Gsndhtsamt., Biol. Abt., Bd. 1, p. 177–222.

(7) HOAGLAND, D. R. 1919 Relation of the nutrient solution to composition and reaction of cell sap of barley. *In* Bot. Gaz., v. 68, p. 297–304.

(8) KRAUS, E. J., AND KRAYBILL, H. R. 1918 Vegetation and reproduction with special reference to the tomato. Ore. Agr. Exp. Sta. Bul. 149.

(9) LAURENT, E. 1891 Recherches sur les nodosites radicales des legumineuses. *In* Ann. Inst. Pasteur, t. 5, p. 105–139.

(10) MAZE, P. 1898 Les microbes des nodosites des legumineuses. *In* Ann. Inst. Pasteur, t. 12, p. 1–26.

(11) McCOOL, M. M., AND MILLAR, C. E. 1917 The water content of soil and the composition and concentration of the soil solution as indicated by the freezing-point lowerings of the tops and roots of plants. *In* Soil Sci., v. 3, p. 113–138.

(12) NEDOKVOCHAYEV, N. 1904 Conditions of accumulation and reduction of nitrates in plants. *In* Izv. Moscov. Selsk. Khoz. Inst. (Ann. Inst. Agron. Moscou), v. 10, no. 1, p. 257–347. *Abs. in* Exp. Sta. Rec., v. 16, p. 1053.

(13) PETERSON, W. H. 1914 Forms of sulfur in plant material and their variation with the soil supply. *In* Jour. Amer. Chem. Soc., v. 36, p. 1290–1300.

(14) PITZ, W. 1916 Effect of elemental sulfur and of calcium sulfates on certain of the higher and lower forms of plant life. *In* Jour. Agr. Res., v. 5, p. 771–780.

(15) PRUCHA, M. J. 1915 Physiological studies of *Bacillus radicicola* of Canada field pea. N. Y. (Cornell) Agr. Exp. Sta. Mem. 5.

(16) SCHULZE, E. 1897 Ueber das Vorkommen von Nitraten in Keimpflanzen. *In* Ztschr. Physiol. Chemie, Bd. 22, p. 82–87.

(17) STEWART, GUY R. 1918 Effect of season and crop growth in modifying the soil extract. *In* Jour. Agr. Res., v. 12, p. 311–368.

(18) TRUOG, E., AND MEACHAM, M. R. 1919 Soil acidity: Its relation to the acidity of the plant juice. *In* Soil Sci., v. 7, p. 469–474.

(19) WILSON, J. K. 1917 Physiological studies of *Bacillus radicicola* of the soybean and its relation to nodule production. N. Y. (Cornell) Agr. Exp. Sta. Bul. 386.

(20) WOO, M. L. 1919 Chemical constituents of *Amaranthus retroflexus*. *In* Bot. Gaz., v. 68, p. 313–344.

THE CAPILLARY POTENTIAL AND ITS RELATION TO SOIL-MOISTURE CONSTANTS

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Although a slight change in the structure of a given soil may appreciably change the value of the potential with constant moisture content, we may nevertheless make use of this physical character to advantage in soil-moisture studies. Buckingham (2) has outlined its significance in the study of moisture movement but recent literature is devoted largely to another point of view.

The potential may involve other factors but obviously a soil which has been allowed to approach a condition of permanent porosity by processes akin to cropping may be characterized by a capillary potential, the magnitude of which is determined by the moisture content and the concentration of dissolved substances.

Briggs (1) has given some preliminary data obtained from a series of soils by use of the "centrifugal" machine, showing that for a considerable range the moisture content ρ is a linear function of the reciprocal of the "centrifugal" force with which it is in equilibrium. If this force is proportional to the potential φ for the corresponding moisture content, the potential is evidently an hyperbolic function of the moisture content over this range, the effective radius r of the soil particles entering as a parameter. This subject has not, however, been exhaustively investigated and it is not unlikely that the function is somewhat more involved.

Whatever may be its form, it is of interest to note that a new interpretation of soil-moisture constants is made available through a consideration of the potential function. The so-called hygroscopic coefficient h, regarded as a function of the soil texture, defines an equipotential curve over the $\varphi\rho r$ surface; and, in a similar manner we may regard the Hilgard-Briggs moisture-holding capacity c, the saturation constant s, and the Briggs moisture equivalent e as specifications of particular equipotential curves. The wilting coefficient w may involve other factors such as operate to influence the *movement* of soil moisture, although it will no doubt define a curve of approximately constant potential. The same may be true of Greaves' (4) biological constants, m_s, m_n, m_{aa}.

The curves of figure 1 have been drawn to illustrate the projection into the $\varphi\rho$ plane of a series of Briggs' curves for different kinds of soil. As stated, he has plotted the reciprocal of the "centrifugal" force against the moisture con-

tent and gets a series of straight lines, whereas, the curves of figure 1 would perhaps more nearly represent the potential curves. A system of concurrent straight lines were drawn and the equipotential lines e, w, and h so located as to give a system of values for the ordinates at the points of intersection with the curves of the family satisfying Briggs' system of linear equations. These curves were extended in such a way as to approximate a family of hyperbolae intersecting the vertical line c at points the ordinates of which would also satisfy these equations.
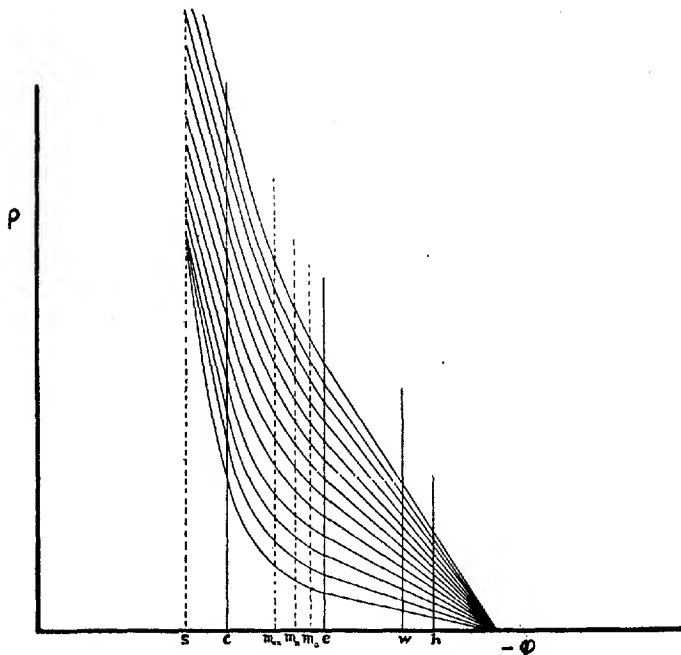
FIG. 1. A SYSTEM OF CURVES SATISFYING BRIGGS' LINEAR EQUATIONS AND CONSISTENT WITH HIS DATA RELATING THE MOISTURE CONTENT TO THE RECIPROCAL OF THE EQUILIBRIUM CENTRIFUGAL FORCE

A comparatively simple transformation of the points on these curves parallel to the φ axis is no doubt possible which would give a family of hyperbolae. It is evident, however, that the lines must remain concurrent at a finite point corresponding to the "adhesion" potential which would perhaps be the same for all soils.

A series of vertical lines have been drawn to illustrate the projection into the $\varphi\rho$ plane of the several equipotential curves indicated above. The ordinates at the various points of intersection would represent the various constants

for the series of soils represented, and it may be readily seen that a knowledge of the functional relation between the potential, the moisture content, and the effective radius of the soil particle, whether obtained empirically or by theoretical speculation, would not only be equivalent to a knowledge of the various moisture constants but also relations that may exist between them such as the Briggs system of equations (3).

Attention should be called to the fact that the function may not be single-valued. It is not difficult from a careful consideration of the surface configuration of the moisture in the soil to conceive of an abrupt change from positive to negative curvature at a given moisture content due to an abrupt uniting of the numerous individual droplets about the points of contact of the soil particles, resulting in a new surface configuration of reverse curvature. The potential would be directly determined by the curvature and two values may thus be possible for a given moisture content over a certain region. In fact, it seems quite impossible to account for a hygroscopic coefficient on any other basis.

Fortunately, a simple method of experimentally determining the potential function is available, as suggested by Buckingham (2). It is perhaps quite immaterial where the zero potential is placed and also what convention is adopted as to the algebraic sign, although it is somewhat more in accord with modern usage to define the potential as the work done by the field forces in bringing unit mass from the point in question to infinity, and in such case the heat of vaporization corresponding to the potential at *s* on the diagram should be added to Buckingham's potential and the negative sign should be used.

In conclusion, it may be stated that a consideration of the subject of soil moisture from the standpoint of the capillary potential gives a new interpretation of the various soil moisture constants. From experimental data already available, it is evident that the potential function may be comparatively simple. The curves which have been plotted are given as indicative of what may be expected although further experimental investigation may suggest a transformation parallel to the potential axis. They are consistent with the well known Briggs equations and with the linear character of his moisture-centrifugal force curves, although, as stated, they are to be regarded as suggestive only, and this article has been written with the hope of emphasizing the point of view rather than to attempt to specify correctly the character of the function.

REFERENCES

(1) BRIGGS, L. J., AND McLANE, J. W. 1907 The moisture equivalent of soils. U. S. Dept. Agr. Bur. Soils Bul. 45.

(2) BUCKINGHAM, EDGAR 1907 Studies of the movement of soil moisture. U. S. Dept. Agr. Bur. Soils Bul. 38.

(3) GARDNER, WILLARD 1919 Capillary moisture-holding capacity. *In* Soil Sci., v. 7, no. 4, p. 324. (Briggs' equations transformed with notation as in this article.)

(4) GREAVES, J. E., AND CARTER, E. G. 1920 Influence of moisture on the bacterial activities of the soil. *In* Soil Sci., v. 10, p. 361-387.

INFLUENCE OF MOISTURE ON THE BACTERIAL ACTIVITIES OF THE SOIL

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Most of the changes which take place in soil are wrought by micro-organisms. They bring about the transformations through which nitrogen passes into the soil, that is to say, the transformation from its organic compounds of the soil or its free form of the atmosphere to a form available to the growing plant. Furthermore, bacteria play an essential part in the cycles through which hydrogen, sulfur and carbon pass. They bring about the mineralization of calcium, iron, phosphorus and other inorganic elements of the plant and animal residues in the soil. Moreover, many compounds are changed from insoluble to soluble forms and thus made available to the growing plant. At times bacteria have an opposite effect and render many of these substances insoluble, thus preventing their loss from the soil through leaching. Or at times they may even compete with the higher plants for the limited supply of nutrients in the soil.

The speed with which these transformations proceed is governed to a marked extent by the water which the soil contains and it is not unlikely that the optimum moisture content of a soil for the production of maximum crops is that water content which is ideal for beneficial bacterial activity of that specific soil. Although investigators have determined the optimum moisture content for the various bacterial activities, yet none have made an attempt to obtain a general expression to cover specific bacterial activities in widely different soils. This investigation was therefore undertaken with the hope of finding some general law governing the water requirements of the various bacteria of the soil and correlating this with the requirements of higher plants.

A careful review of the literature dealing with the various phases of the subject has been made, and there is given under each division a resumé of the most important.

SOILS

The soils used in the work were collected during the fall of 1919. They represent 22 different farms, and all except three were from Cache Valley, taken within a radius of 45 miles within the basin of what used to be Lake Bonneville. They are of sedimentary origin and came from the nearby

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mountains which are composed largely of quartzite and limestones. All are well supplied with phosphorus, potassium and other essential elements (49), with the exception of humus and nitrogen which in the majority of cases is low, as is characteristic of the soils of arid America. Some of the soils con-

TABLE 1

Soils used in determining moisture requirement of bacteria

SAMPLE NUMBER	TYPE OF SOIL	LOCATION	CROP	TREATMENT
1	Clay loam	J. C. Johnson Farm, North Logan	Beets—8 years	Irrigated, manured
2	Clay loam	J. C. Johnson Farm, North Logan	Beets—8 years	Irrigated, manured
3	Tight clay loam	J. C. Johnson Farm, North Logan	Alfalfa—2 years	Irrigated, manured
4	Sand loam	North Logan	Weeds	Irrigated, manured
5	Light sand loam	Jacob Swartz Farm, E. North Logan	Wheat—2 years	Dry land
6	Clay loam	Parley Armond Farm, North Logan	Wheat	Irrigated
7	Peaty loam (alkali)	R. Smith Farm, West Logan	Pasture	Irrigated
8	Silt loam	East Petersboro	Wheat, continually	Dry land
9	Black loam	Miller Bros. Farm, E. Petersboro	Wheat	Dry land
10	Very tight clay	Kidman Farm, Petersboro	Barley	Dry land, no manure
11	Silt loam	Near Mendon	Wheat—8 years	Dry land, no manure
12	Extra tight clay	Near Mendon	Barley	Dry land, no manure
13	Trenton fine loam	Sugar Spur, W. Logan (McCombs)	Beets—3 years	Irrigated, manured
14	Fine silt loam (alkali)	Johnson Farm, West Logan	Beets—2 years	Irrigated, no manure
15	Light sandy loam	Providence (Hanson Farm)	Beets—15 years	Irrigated, manured
16	Medium sand loam	Providence (Allen Farm)	Corn	Irrigated, manured
17	Sand	Providence Bench	Nothing	Dry land
18	Light mountain loam	Bothwell, Utah (Sorenson Farm)	Wheat	Dry land
19	Loose, light mountain loam	Johnson Farm, Blue Creek	Wheat—4 years	Dry land
20	Fine sand	Hansen Farm, Garland	Fallow, wheat	Dry land
21	Organic loam	Hansen Farm, Collinston	Wheat—12 years	Dry land
22	White clay loam	Poulson Farm, Collinston	Wheat—10 years	Dry land

tain as high as 50 per cent of calcium and magnesium carbonate. They are all remarkably fertile and produce excellent crops for many years without the addition of manures. Although quite similar they possess a great variation in physical properties. Most of the soils were ideally adapted both chemically and bacteriologically to support rapid bacterial action.

The type, location, crop and general treatment of the various soils are given in table 1.

These soils represent the typical farming lands of Cache Valley—dry land, irrigated, manured, and unmanured. Their treatment has been greatly varied and although we give here the crop for only a few years, yet some of them have been tilled for more than fifty years.

They range all the way from the loose sand, as represented by no. 17, to the tight clay of no. 12, as may be seen from table 2.

TABLE 2

Physical analysis of soils used in determining moisture requirement of bacteria

SAMPLE NUMBER	MEDIUM SAND	FINE SAND	COARSE SILT	MEDIUM SILT	FINE SILT	CLAY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	27.544	35.966	14.121	9.972	6.173	7.083
2	21.001	44.250	21.086	11.087	2.098	5.240
3	43.676	53.805	19.521	10.674	4.327	9.101
4	18.282	33.085	19.804	12.785	9.340	6.983
5	26.884	27.270	21.421	11.403	7.443	7.715
6	4.500	26.487	27.905	17.955	9.974	11.363
7	6.049	31.578	26.077	18.594	7.767	14.450
8	12.501	21.963	20.260	15.195	11.137	11.033
9	8.010	24.867	24.176	20.441	10.245	16.136
10	3.630	19.502	25.020	24.785	18.837	12.642
11	15.560	33.799	16.928	14.764	9.948	11.313
12	1.086	6.363	17.866	27.381	26.227	19.896
13	20.806	41.067	19.133	9.025	6.015	7.209
14	17.267	30.072	22.536	16.375	3.685	2.265
15	77.882	9.374	5.539	3.566	3.035	4.841
16	14.577	35.581	23.345	13.576	6.690	7.485
17	90.140	7.941	1.252	0.703	0.443	0.831
18	39.965	30.275	13.751	5.377	5.010	7.409
19	28.252	39.064	11.774	8.822	5.860	6.302
20	89.104	4.571	1.539	1.925	1.754	2.079
21	13.074	26.350	23.957	14.794	12.510	1.018
22	20.129	31.338	15.749	12.645	8.195	13.340

The analysis was made by means of the Yoder elutriator (53), and the different separates are those indicated by him. The medium sand ranges from 1.086 in no. 12 to 90.14 in no. 17. The fine sand varies from 4.571 in no. 20 to 53.805 in no. 3, the coarse silt from 1.252 in no. 17 to 27.905 in no. 6, medium silt from 0.703 in no. 17 to 24.785 in no. 10, fine silt from 0.443 in no. 17 to 26.227 in no. 12, and the clay from 0.831 in no. 17 to 19.896 per cent

in no. 12. Thus we have a wide variation in so far as physical composition is concerned and hence their bacterial analysis should indicate the different moisture requirements of various soils.

This was determined by the method devised by Hilgard and modified by Briggs (37). Small cups 5 cm. in diameter and 1 cm. in height with the bottom made of fine screen were used. The soil was settled slightly by jarring and stroked off level with the top of the cup. The filled cups were then placed with the bottom in water, and when the soil had taken up their maximum amount of water they were allowed to drain for 30 minutes. The percentages of moisture in the soil were then determined by weighing before and after drying.

The average results for four closely agreeing determinations are given in table 3.

TABLE 3

Maximum water-holding capacity of soil

NUMBER	WATER-HOLDING CAPACITY	NUMBER	WATER-HOLDING CAPACITY
	per cent		*per cent*
17	31	10	61
20	33	12	61
15	44	2	61
18	51	4	62
13	51	3	65
5	53	1	68
11	56	7	74
19	56	8	77
6	57	14	78
9	59	21	78
16	60	22	78

This gives us a set of soils with a water-holding capacity of from 31 to 78 per cent. It is quite evident from these results that the water-holding capacity of these soils is due in the main to the quantity of clay and organic matter. Soil 17 has the lowest water-holding capacity and it also has the lowest percentage of clay. Soil 21, with the highest water-holding capacity, is next in clay content, but it possesses more organic matter than any of the other soils.

The ammonifying, nitrifying, and nitrogen-fixing powers of the soil with the various moisture contents were determined as follows:

The ammonifying power of the soil was determined by weighing 100-gm. portions of the soil and 2 gm. of dried blood into sterile tumblers and covering

them with petri dishes. The dried blood was thoroughly mixed with the soil by means of a sterile spatula and the water content made up to from 10 to 100 per cent in 10 per cent increments of their water-holding capacity. The samples were incubated at 28° to 30°C. for 4 days and the ammonia determined by transferring to Kjeldahl flasks with 400 cc. of distilled water, adding 2 gm. of magnesium oxide and distilling into 0.1 N sulfuric acid.

The nitrifying power of the soils was determined in tumblers similar to the determination of the ammonifying power, except that they were incubated for 21 days. The moisture content was made up every 3 days to the initial percentages, and the nitric nitrogen determined as follows (18).

The contents of the beaker, together with 500 cc. of distilled water and 2 gm. of alum, were placed in quart Mason jars and agitated for 5 minutes in a shaker.

An aliquot part (100 cc.) of the supernatant liquid was pipetted off, and, together with 2 cc. of a saturated solution of sodium hydroxide, was evaporated to about one-fourth of its original volume to free it from ammonia. To this were added 50 cc. of ammonia-free water, 5 gm. of "iron-by-hydrogen," and 30 cc. of sulfuric acid (sp. gr. 1.35). If less than 40 mgm. of nitric nitrogen is to be determined, it is well to take a correspondingly smaller quantity of iron and sulfuric acid. The neck of the reduction flask was fitted with a 2-hole stopper, through which passed a 50-cc. separatory funnel and a bent tube which dipped into a vessel containing water in order to prevent mechanical loss. The acid was slowly added and allowed to stand until the rapid evolution of hydrogen was over. It was then heated to boiling for 10 minutes. The contents of the side vessel were returned to the reduction flask before the reaction was complete, thus insuring the complete reduction of any nitrates which may have been carried over with the first violent evolution of the hydrogen. The contents of the reduction flask were transferred to Kjeldahl flasks, neutralized with sodium hydroxide, and distilled into standard acid. The excess of acid was titrated back with standard alkali, lacomoid being used as an indicator. Controls were run on all the reagents including the alum used as a flocculant.

The nitrogen-fixing powers of the soil were determined by weighing 100 gm. of soil and 1.5 gm. of lactose into sterilized tumblers covered with petri dishes. Sufficient sterile distilled water was added to bring the moisture up to the required percentage. The samples were incubated for 21 days at 28° to 30°C. The moisture content was made up to the required content every other day. The tumblers and contents at the end of the incubation period were dried at 95°C., ground in a mortar and 20-gm. portions used for the determination of nitrogen by the Gunning method revised to include nitrates (21).

In the ammonifying, nitrifying and nitrogen-fixing work five or six determinations were made at each water content and the results as reported, throughout this work are the averages of a number of closely agreeing determinations.

Ammonifying powers. The influence of moisture on the ammonia found in the soil is very great. Lipman and Brown (32) found ammonification in a loam soil to increase with increased water content even up to 35 per cent of the weight of the soil. However, later they and Owen (33) found ammonification to increase as the water added increased up to a certain percentage, which varied with the physical nature of the soil, but larger quantities of water reduced the ammonia recovered. The work clearly demonstrated that the optimum moisture content for maximum ammonification is higher than it is for maximum nitrification. The quantitative difference between the two processes in the same soil was found by Sharp (44). Ammonification was most rapid with a 25 per cent moisture content and was not markedly affected by 3 per cent differences. Nitrification was at its maximum when the soil contained 19 per cent of water. When it was increased to 25 per cent the rate of nitrification was decreased 50 per cent.

When soils are held at a certain moisture content for several months and then all brought to a corresponding moisture content (20 per cent) and the ammonia determined after 4 days, the variation in moisture content affects very materially the ammonia produced, as seen from the following results obtained by Greaves and Carter (17):

MOISTURE ADDED	PER CENT OF AMMONIA PRODUCED
12.5 per cent of water..	100
15.0 per cent of water..	111
17.5 per cent of water..	113
20.0 per cent of water..	123
22.5 per cent of water..	119

This increased ammonification with increased moisture content is due, according to Lipman, to the suppression of the aerobic decay bacteria and an acceleration of the anaerobic putrefactive bacteria.

Robson (41) studied the changes produced in the nitrogen compounds in the natural organic matter of soils and ammonium sulfate and horn meal in sandy loam and clay soils with varying amounts of water (6, 12 and 18 per cent in sandy soil; 8, 16 and 24 per cent in loam; and 8, 18 and 28 per cent in clay). With a low moisture content the transformations of organic nitrogen were more rapid in sandy soils than in the heavy soils, whereas with a higher moisture content there was little difference.

The work at the Utah Agricultural Experiment Station (20) demonstrated that the application of water to a soil increased its ammonifying powers, as is shown in the following:

per cent

No water...	100
15.0 inches of water...	103
25.0 inches of water...	97
37.5 inches of water...	104

The soil which received no irrigation water in these plats was taken as producing 100 per cent of ammonia.

The results which we have obtained in the study of the ammonifying powers of the 22 soils previously described are given in table 4. The water contents of the soil were 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 per cent of its water-holding capacity. The results as reported are the averages of four or more closely agreeing determinations.

TABLE 4

Ammonia formed in 100 gm. of soil maintained at various moisture contents expressed in per cent of water-holding capacity

NUM-BER	KIND OF SOIL	AMMONIA FORMED									
		10 per cent	20 per cent	30 per cent	40 per cent	50 per cent	60 per cent	70 per cent	80 per cent	90 per cent	100 per cent
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	Clay loam	0.3	28.0	51.0	65.0	76.0	135.0	127.0	72.0	71.0	69.0
2	Clay loam	2.0	20.0	39.0	91.0	97.0	109.0	63.0	54.0	49.0	57.0
3	Tight clay loam	0.3	2.0	34.0	83.0	101.0	127.0	90.0	67.0	60.0	51.0
4	Sand loam	0.2	5.0	14.0	53.0	58.0	71.0	57.0	55.0	63.0	55.0
5	Light sand loam	0.2	3.0	32.0	61.0	72.0	75.0	67.0	43.0	40.0	30.0
6	Clay loam	0.3	1.0	5.0	14.0	27.0	33.0	30.0	27.0	26.0	25.0
7	Peaty loam	2.0	3.0	6.0	28.0	34.0	58.0	48.0	31.0	29.0	24.0
8	Silt loam	0.4	2.0	21.0	55.0	67.0	78.0	52.0	28.0	25.0	25.0
9	Black loam	0.0	0.3	0.6	48.0	80.0	100.0	88.0	56.0	4.0	3.0
10	Very tight clay	1.0	2.0	4.0	44.0	55.0	69.0	63.0	36.0	40.0	32.0
11	Silt loam	2.0	3.0	14.0	53.0	55.0	71.0	37.0	36.0	33.0	11.0
12	Extra tight clay	1.0	2.0	2.0	2.0	8.0	13.0	12.0	12.0	11.0	9.0
13	Trenton fine loam	1.0	2.0	32.0	70.0	94.0	126.0	124.0	104.0	75.0	60.0
14	Fine silt loam	4.0	6.0	20.0	60.0	62.0	86.0	44.0	45.0	45.0	41.0
15	Light sandy loam	0.9	14.0	42.0	61.0	74.0	109.0	103.0	39.0	39.0	36.0
16	Medium sand loam	0.0	0.4	9.0	31.0	41.0	50.0	48.0	44.0	39.0	41.0
17	Sand	0.7	19.0	23.0	40.0	57.0	74.0	71.0	51.0	41.0	30.0
18	Light mountain loam	3.0	16.0	52.0	109.0	126.0	127.0	100.0	61.0	55.0	55.0
19	Loose, light mountain loam	3.0	6.0	88.0	115.0	123.0	126.0	79.0	50.0	47.0	44.0
20	Fine sand	0.5	21.0	35.0	47.0	64.0	72.0	42.0	39.0	31.0	32.0
21	Organic loam	2.0	3.0	48.0	93.0	107.0	116.0	96.0	72.0	55.0	41.0
22	White clay loam	3.0	5.0	38.0	51.0	70.0	81.0	46.0	46.0	47.0	47.0
	Average	1.3	7.4	28.0	57.9	70.4	86.6	67.6	48.6	42.0	38.1

The soils show a wide variation in ammonifying powers. Although the quantity of sand present has a marked effect upon ammonification yet the predominating factor in these soils is the quantity of organic matter present. Those soils which had received a heavy dressing of manure are the ones which showed the most active bacterial changes. No. 12, the extra tight clay, never produced over 13 mgm. of ammonia, whereas no. 1, a clay loam, heavily manured, produced 135 mgm.

The quantity of ammonia produced when the water added is 10 per cent *of the soil's water-holding capacity is very low in each case. When the water is raised to 20 per cent, ammonification becomes quite active in the lighter soils, but in the heavier soils there is a very slight increase.* There is a gradual increase in ammonification as the water added increased up to 60 per cent. At this point every soil gives its maximum ammonification. The great variation in the physical composition of the soils and the large number of determinations which have been made would render it quite certain that maximum ammonification occurs in soils which contain water equal to 60 per cent of their water-holding capacity. This stated as the per cent of water in the soil would show a marked variation in the different soils, being very low in the case of the sand and high in the case of the organic loam. The lowest would be in the case of soil 17 which produced its maximum quantity of ammonia when it contained 18.6 per cent of water, whereas no. 22 produced its maximum quantity of ammonia when it contained 46.8 per cent of water, or the quantity of water necessary to add to these two soils is over three times as much in one case as it is in the other for maximum ammonification. This would account for the marked differences reported by various investigators as to the water required by ammonifying bacteria in soil.

As the water added increases above 60 per cent there is a gradual decrease in ammonification. In no case is this abrupt and even in saturated soils there is rapid ammonification. Considering the maximum ammonia produced at 60 per cent of water, the quantity produced at the other moisture contents is shown in figure 1.

Nitrification. Long before the process of nitrification was known to be due to microörganisms the underlying principles governing the speed of the reaction had been investigated nationally by France, Germany and Sweden. Among other things, they had learned that there must be a certain proportion of water, and in order that the maximum yield of nitrates be obtained this must be diminished as the soil becomes richer in nitrates. As early as 1887 Deherain (6) found that the most active nitrification took place when the soil was allowed to become partially dry between the applications of water, and later (7) he found that there was a relationship between the speed of nitrification and the moisture content of fallow soil, the nitrification increasing with the water. Boussingault (47) taught that when soils contain as much as 60 per cent of water they lose in a few weeks the greater part of their nitrates. This teaching gave rise to the general belief that denitrification may take place to a great extent in soils, but recent work has amply demonstrated that it is only under extremely abnormal conditions that this becomes an important factor. For this reason literature bearing on this phase of the subject is not considered here.

Deherain and Demoussy (9) found that the bacterial action of a soil was at its maximum when a rich soil contained 17 per cent of water, but that it decreased if the proportion of water fell to 10 per cent or rose to 25 per cent.

With soils less rich in humus a somewhat higher proportion of water was necessary to retard oxidation to any marked degree.

The optimum moisture content for nitrification, according to Deherain (7), is 25 per cent. An insufficient supply of moisture checked both nitrification and nitrogen fixation. This occurred when the water had been reduced to
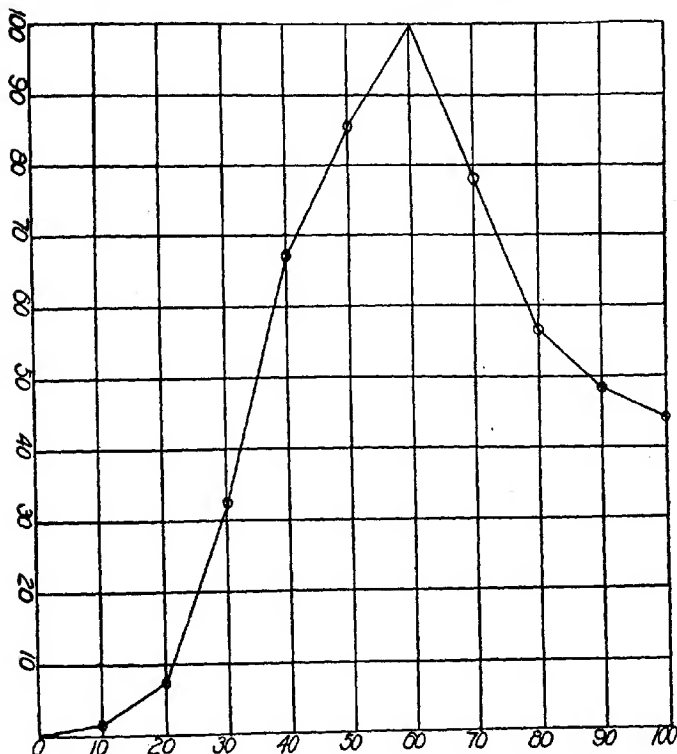
FIG. 1. AVERAGE PERCENTAGES OF AMMONIA PRODUCED IN SOILS RECEIVING VARYING
QUANTITIES OF WATER.

The quantity produced at 60 per cent is taken as 100; on the ordinate is given the per cent of ammonia formed, whereas on the abscissa is given water applied as per cent of water-holding capacity.

16.5 per cent. This, however, would vary with the soil, for Schloesing (42) found bacterial activity less in fine-grained soils than in lighter, coarse-grained soil. In order that nitrification be equally active in both light and heavy soils the latter must have a higher percentage of water than the former, a difference in moisture content of the soil of 1 per cent, according to Dafert

and Bollinger (4), being sufficient to produce a marked change in the oxidation going on in the soil.

Fraps (13) found that the number of nitrifying organisms in a soil varies with the moisture and that their activity is periodic, rapid nitrification being preceded and followed by periods of less activity. Later he (14) found nitrification to be at its height in soil containing 55.6 per cent of its water-holding capacity. An excessive quantity of water practically stopped nitrification and was much more injurious than too small a quantity. The water requirements, however, varied considerably with the soil.

Coleman's work (3) with a loam soil showed nitrification to be most active when the soil contained 16 per cent of water. It was greatly retarded when the water content was reduced to 10 per cent or increased to 26 per cent. Not only nitrification but ammonification is dependent upon the moisture content of the soil. However, Lipman and Brown (32) found that ammonification in a loam soil increased with an increased water content even up to 35 per cent of the weight of the soil, but nitrification was most active in the same soil with a moisture content of 15 per cent, was only slightly less active with 10 per cent of moisture, and was still quite marked when the soil contained only 5 per cent of moisture. They found the greatest formation of nitrates to occur in soils when they were 50 per cent saturated with water. However, later Lipman, Brown and Owen (33) found ammonification to increase as the water added increased up to a certain percentage, which varied with the physical nature of the soil; but larger quantities of water reduced the ammonia recovered. Moreover, the work clearly demonstrates that the optimum moisture content for maximum ammonification is higher than it is for maximum nitrification.

Engberding (10) considered that the moisture content of a soil had a greater influence on numbers than did temperature, and the work of King and Doryland (27) clearly indicates that excessive soil moisture reduces both the number and the activity of soil bacteria.

Patterson and Scott's work (40) is interesting in that they found nitrification to be inactive in sand and clay soils which still contained about three times as much moisture as in their average air-dry condition. At the lower limits of moisture less water starts nitrification in sand than in clay. At the higher limits of moisture less water stops nitrification in sand than in clay, while the optimum amount of water probably varies for each soil. It is higher for clay, yet for both soils it lies within the range of from 14 to 18 per cent. A rise above the optimum amount of water is more harmful than an equal fall below it.

The work of the Utah Experiment Station (45) demonstrated that the application of irrigation water to a soil has a distinct beneficial effect upon nitrification, being greatest where 15 inches of water were applied when the nitric nitrogen formed amounted to 28.5 pounds per acre-foot of soil. The greatest benefit per inch of water, however, was obtained where only 7.5

inches of water were applied, resulting in 3.8 pounds of nitric nitrogen per inch of water, while where 15 inches of water were applied it was 1.1 pounds of nitric nitrogen per inch of water applied, and when 25 inches of water were applied to the soil the nitric nitrogen produced was only 0.7 pound.

Münter and Robson (38) found that horn meal decomposed more rapidly in dry, sandy soil than in clay or loam, while with a higher moisture content there was little difference. Ammonium-sulfate transformation increased with a higher water content. The best nitrate formation from horn meal occurred in sandy soils. In clay and loam it was best with a medium water content. Sharp (44) found that the water content most favorable for ammonification was not the optimum condition for nitrification. The former was most rapid with a 25 per cent water content and was not markedly affected by 3 per cent differences. Nitrification was at its maximum when the soil contained 19 per cent of water. When it was increased to 25 per cent, the rate of nitrification was decreased 50 per cent.

McBeth and Smith (36) found a slight variation in the bacterial number and nitrifying power of soils, depending upon the moisture content. However, Gainey (15) considers that among the factors controlling the bacterial activity of a soil the available moisture probably plays a leading part. But we (19) have reported results which indicate that the nitrous-nitrogen content of a soil is independent of the irrigation water applied up to 37.5 inches a year. Results recently published from the Utah Experiment Station (17) clearly demonstrate that the influence exerted by water upon ammonifying, nitrifying, and nitrogen-fixing activities of the soil varies greatly with the organic matter in the soil and is much more marked in effect on soils recently manured than on those which have received no manure.

However, in tests of soil receiving varying quantities of water in the field (20) no increase of nitric-nitrogen accumulation or increased nitrification was noted, as is seen from the following:

TREATMENT	NITRIC NITRO-GEN IN SOIL	NITROUS NITRO-GEN IN SOIL	NITRIFYING POWERS
	per cent	*per cent*	*per cent*
No water..................................	100	100	100
15.0 inches................................	48	115	98
25.0 inches................................	51	62	98
37.5 inches................................	43	115	98

The decrease in nitric nitrogen may be due to a number of factors: (1) washing out by the water, (2) increased plant growth and hence the use of more nitrates, (3) increased bacterial activity which would transform nitrates into proteins. The figures in the third column would indicate that although the addition of water to a soil changes its nitrifying powers it is not permanent.

The same set of soils used in the previous section to determine the influence of water on the ammonifying power was used to study the nitrifying power.

The water varied from 10 to 100 per cent of the water-holding capacity of the soil. The results, as given in table 5, are the average of six of more closely agreeing determinations.

The soils show a wide variation in their nitrifying power, yet all are quite active, as would be expected of them since they are well supplied with potassium, phosphorus, and other elements essential to bacterial growth, with the exception of nitrogen. This element in the case of the unmanured soils is

TABLE 5

Nitric nitrogen formed in 100 gm. of soil maintained at various moisture contents expressed in per cent of water-holding capacity

SAMPLE NUMBER	KIND OF SOIL	NITRIC NITROGEN FORMED							
		10 per cent	20 per cent	30 per cent	40 per cent	50 per cent	60 per cent	70 per cent	80 per cent
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	Clay loam............	10.2	16.5	28.0	60.5	65.2	85.3	7.5	4.0
2	Clay loam............	0.4	7.8	21.5	32.0	38.0	17.5	0.8	1.2
3	Tight clay loam.......	1.0	3.9	10.8	58.0	92.3	76.4	2.4	2.2
4	Sand loam............	3.6	6.1	20.7	26.0	81.1	94.1	7.4	4.6
5	Light sand loam.......	3.7	7.0	11.7	21.9	51.6	20.0	15.7	3.1
6	Clay loam............	2.9	4.3	10.0	34.7	77.5	105.2	38.1	2.9
7	Peaty loam...........	0.0	0.4	5.5	31.9	72.2	142.7	36.9	3.7
8	Silt loam.............	6.5	11.2	25.0	67.8	89.7	73.8	11.6	2.4
9	Black loam...........	3.5	5.0	12.0	27.2	45.8	52.4	49.9	7.1
10	Very tight clay........	6.0	8.4	14.1	60.1	59.4	99.7	40.2	4.0
11	Silt loam.............	3.5	9.2	34.0	59.9	60.4	61.5	27.0	2.2
12	Extra tight clay.......	10.9	11.6	11.8	11.7	12.3	3.0	3.0	3.7
13	Trenton fine loam......	12.1	18.4	39.2	60.3	79.4	65.9	29.8	13.8
14	Fine silt loam.........	23.9	24.5	27.7	28.9	39.2	121.4	57.4	18.1
15	Light sandy loam......	17.0	25.2	25.5	25.7	26.4	20.3	19.2	18.3
16	Medium sand loam.....	0.7	2.2	10.6	16.8	29.2	101.6	90.4	2.5
17	Sand.................	2.8	3.8	4.0	5.9	6.8	3.6	2.4	2.1
18	Light mountain loam...	6.3	13.3	27.0	51.4	53.8	26.1	2.5	2.3
19	Loose, light mountain loam...............	4.4	8.9	10.6	11.6	24.0	25.0	6.0	4.3
20	Fine sand.............	6.0	5.6	5.6	5.4	4.3	3.5	3.6	3.6
21	Organic loam.........	3.8	6.9	18.7	40.7	56.0	56.7	40.7	9.4
22	White clay loam.......	8.5	13.2	12.7	42.7	20.6	6.1	5.9	6.0
Average		6.3	9.7	17.6	35.5	49.3	57.4	22.7	5.5

low. All contain large quantities of calcium and magnesium carbonate. As an average, those soils which showed a low ammonifying power also show a comparatively low nitrifying power. It is interesting to note that nitrification is comparatively more active with the 10 per cent of water than is ammonification. However, the addition of water to the soil greatly accelerates nitrification. Although the quantity of nitric nitrogen accumulating in the soil progressively increases with increasing water up to a certain point, yet it

is much more pronounced in some soils than in others. About half of the soils produce maximum quantities of nitric nitrogen at 50 per cent saturation, whereas the others produce most at 60 per cent. This probably indicates that optimum moisture for maximum nitrification in soils lies somewhere between 50 and 60 per cent of saturation, thus indicating, as has been
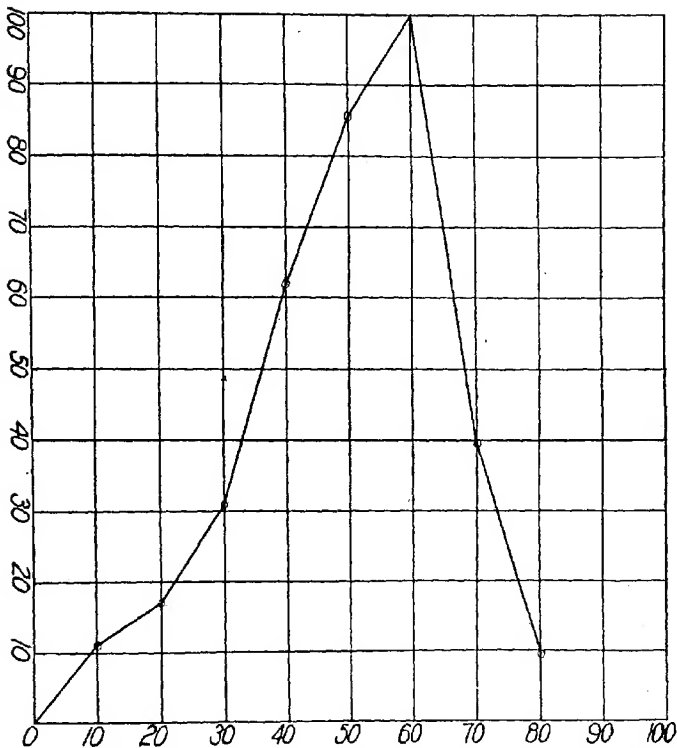
FIG. 2. AVERAGE PERCENTAGES OF NITRIC NITROGEN PRODUCED IN SOIL RECEIVING VARIOUS QUANTITIES OF WATER

The quantity produced at 60 per cent is taken as 100; on the ordinate is given the per cent of nitric nitrogen formed, whereas on the abscissa is given water applied as per cent of water-holding capacity.

the finding of many other workers, that the optimum moisture content of soil for best nitrification is somewhat lower than is required for optimum ammonification. When this is considered from the viewpoint of water-holding capacity the results are remarkably uniform as compared with the results

reported by most previous workers. These results, therefore, make it clear that the more logical method is to consider moisture from the standpoint of water-holding capacity of the soil and not from its percentage in the soil. The average results are given graphically in figure 2.

When the water content of the soil exceeds 60 per cent there is a rapid decrease in the nitric-nitrogen content. All soils ceased to nitrify when saturated; it was very slight at the 90 per cent of the water-holding capacity and in most soils less nitric nitrogen was produced where the water content was 80 per cent of saturation than where it was 10 per cent.

It is interesting to note the uniform correlation between ammonification and nitrification. Examining the results in tables 4 and 5 we find that wherever ammonification is higher at 70 per cent saturation than it is at 50 per cent the optimum for nitrification in that soil is at 60 per cent of the water-holding capacity and conversely, wherever ammonification is higher at 50 per cent than it is at 70 per cent, the optimum moisture for maximum nitrification is at 50 per cent.

INFLUENCE OF WATER ON NITROGEN FIXATION

Azotobacter are very resistant to drying; they may be dried for a considerable time in a desiccator over sulfuric acid. Pure cultures are just as resistant to drying as are mixed cultures (25). This would vary somewhat with the media in which the bacteria are dried, for the survival of non-spore-forming bacteria in air-dry soil is due in part to the retention by the soil of moisture in the hygroscopic form. This however, is not the only factor, for the longevity of bacteria in a solid is not directly proportional to its grain size and hygroscopic moisture. Giltner and Langworth (16) found that bacteria resisted desiccation longer in a rich clay loam than in sand. Furthermore, if bacteria are suspended in the extract from a rich clay loam before being subjected to desiccation in sand they live longer than if subjected to dessication after suspension in a physiological salt solution. Because of this they consider that soils contain substances which have a protective influence upon bacteria subjected to desiccation.

Lipman and Burgess (30) have found that many soils manifest a vigorous nitrogen-fixing power even after being air-dried and kept in stoppered museum bottles for periods varying from 5 to 20 years. In some cases the fixation was equally as high as in freshly-collected samples. The organisms from such soils are more easily attentuated than are other organisms which have not been so dried (50). The tendency is for soils gradually to decline in nitrogen-fixing power on drying. This may manifest itself as early as the second week.

During the periods of drying the organisms are inactive as they require moisture for growth and reproduction. For maximum nitrogen-fixation a definite moisture content is needed. Warmbold (48) found the optimum

moisture content to be 20 per cent. When it was below 10 per cent there was no nitrogen fixed, and in some cases there was a decided loss of nitrogen. Krainski (28) allowed soil with varying moisture content to stand for some time and then inoculated it into mannite solutions and obtained maximum fixation in the soils containing fairly small quantities of water. Later, however, he decided that soil should be damp—but not wet—and well aerated for maximum nitrogen fixation. The water requirements vary with different soils. As a general rule the higher the humus content of the soil the more water will be required for optimum nitrogen fixation (29). The quantity of water present may, however, become so great that it may kill all *Azotobacter* in addition to stopping nitrogen fixation (11).

An insufficient supply of moisture checks both nitrification and nitrogen fixation (8). This occurs in some soils when the water content has been reduced to 16.5 per cent. This again varies with the soil, for Schloesing (42) found bacterial activity less in fine-grained soils than in lighter, coarse-grained soils. A difference in moisture content of 1 per cent, according to Defert and Bollinger (4), is sufficient to produce a marked change in the oxidation going on in the soil.

The moisture requirement of the nitrogen-fixing bacteria, according to Lipman and Sharp (31) is more nearly that of the ammonifying than of nitrifying organisms. In a sandy loam it was found to vary between 20 and 24 per cent of moisture in the soil. At the higher percentages of moisture up to 24 per cent the anaerobic nitrogen fixers are most active, but the action of the aerobes is slightly depressed. Thus, in many soils two maxima of nitrogen fixation occur, depending upon whether the conditions are favorable for the anaerobic or aerobic organisms.

Traaen's results (46) differ from Lipman's in showing only the one maximum, as is seen from the following which gives the milligrams of nitrogen fixed in 100 gm. of soil.

TEMPERATURE	PER CENT OF WATER				
	5	10	17.5	25	30
°C.	mgm.	mgm.	mgm.	mgm.	mgm.
13	0.1	1.5	11.2	13.4	5.4
25	1.9	1.9	13.2	16.6	15.5

He used a loam soil with a maximum water capacity of 27.4 per cent. It is quite evident from his statement that anaerobic organisms played a prominent part in the fixation at the higher moisture contents.

Since the carbohydrates disappeared much more rapidly in the soils containing the greater quantities of water, it is quite possible that greater quantities of nitrogen per gram of carbohydrate consumed are fixed where the smaller quantities of water are applied. This, together with the different

methods used by the several investigators, would explain the apparent discrepancy in their results.

In a series of pot experiments in which a calcareous loam receiving various amounts of water was used the author (17) found the moisture content for maximum nitrogen fixation to lie between 15 and 22 per cent. These results also brought out the two maxima which were first noted by Lipman. These soils were kept at the various moisture contents for four months. All were then incubated at 28°C. for 21 days with a moisture content of 20 per cent.

TREATMENT	NITROGEN FIXED
per cent of water added	*per cent*
12.5	100
15.0	108
17.5	102
20.0	104
22.5	108

In this soil the optimum for the aerobes would appear to be at 17.5 per cent and that for the anaerobes 22.5 per cent or higher.

When too large a quantity of water is applied there is a tendency to depress the total nitrogen fixed, as is illustrated by the following results in which various quantities of water were applied to a soil throughout the year under field conditions (20).

37.5 inches of water applied during summer; 1.4 mgm. of nitrogen fixed in 100 gm. of soil

25.0 inches of water applied during summer; 2.1 mgm. of nitrogen fixed in 100 gm. of soil

15.0 inches of water applied during summer; 8.5 mgm. of nitrogen fixed in 100 gm. of soil

No water applied during summer; 3.5 mgm. of nitrogen fixed in 100 gm. of soil

The maximum for anaerobic conditions does not appear in these results probably because the soil did not become filled with water, since under field conditions the water rapidly drains away or is evaporated.

The same 22 soils as were used in the ammonifying and nitrification tests were used to study the influence of water on nitrogen fixation. The moisture was kept at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 per cent of the water-holding capacity and the results as given in table 6 are the average of six or more closely agreeing determinations.

There is a wide variation in the nitrogen-fixing powers of the soil. Some are very active, as for instance no. 7, the peaty loam, and no. 21, the organic loam. In reality it would appear that the soils of this series with the greatest quantity of organic matter are most active as nitrogen fixers. One soil, no. 22, the white clay loam, lost nitrogen with each concentration of water. This was uniformly true in the sixty determinations made on this soil. Five

other soils, no. 4, 6, 8, 18 and 19, lost nitrogen with some concentrations of water and gained with the other concentrations. It is interesting to note that the loss is usually at the lower water contents, whereas at higher water contents there is a gain of nitrogen. This gain usually appears when enough water has been added to produce anaerobic conditions in the soil. From this one must conclude that the anaerobes are the principal azofiers in these soils. Only one soil (no. 16) fixed nitrogen with a low water content and lost

TABLE 6

Nitrogen fixed in 100 gm. of soil maintained at various moisture contents expressed in per cent of water-holding capacity

SAMPLE NUMBER	KIND OF SOIL	NITROGEN FIXED									
		10 per cent	20 per cent	30 per cent	40 per cent	50 per cent	60 per cent	70 per cent	80 per cent	90 per cent	100 per cent
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	Clay loam......	2.4	4.5	7.8	8.7	2.8	7.5	4.2	5.9	0.0	−1.4
2	Clay loam......	6.7	4.7	3.9	3.6	11.5	10.3	5.9	3.5	·5.0	4.2
3	Tight clay loam..	4.2	10.9	13.7	19.6	9.2	10.0	12.6	8.9	7.0	9.4
4	Sand loam......	−8.9	−6.4	−6.1	−1.9	−2.1	8.1	10.0	12.0	3.0	0.0
5	Light sand loam.	1.9	5.2	6.7	3.8	2.2	4.9	3.9	12.9	3.6	−1.1
6	Clay loam......	3.1	−3.0	−2.2	0.2	−0.3	1.2	8.4	3.9	−1.4	−2.1
7	Peaty loam.....	9.8	−3.5	−9.8	−3.9	0.0	11.0	17.7	19.6	10.9	2.6
8	Silt loam.......	−12.2	−19.1	−15.1	−14.8	−5.7	−7.3	−1.4	2.2	−4.0	−16.9
9	Black loam.....	0.1	2.1	−0.8	−6.8	−0.2	0.6	3.0	3.0	1.4	2.1
10	Very tight clay..	3.7	2.2	3.4	0.5	2.5	6.9	3.5	5.9	5.6	6.8
11	Silt loam.......	4.6	4.7	−0.1	0.5	3.5	8.1	4.2	8.7	6.0	2.6
12	Extra tight clay..	3.9	0.8	2.0	−0.5	1.3	5.2	2.4	4.8	4.2	5.6
13	Trenton fine loam	2.1	3.0	5.5	5.6	8.2	16.8	16.8	4.6	6.8	5.6
14	Fine silt loam...	9.1	10.8	9.1	1.4	7.2	5.3	7.7	1.4	3.5	4.4
15	Light sandy loam	7.3	9.2	4.4	5.8	11.0	9.5	13.7	5.6	5.8	6.6
16	Medium sand loam.........	3.5	3.8	4.2	5.6	1.4	0.2	5.6	−1.0	−7.5	−6.3
17	Sand..........	7.0	4.2	0.8	0.3	1.4	1.1	0.3	2.1	1.4	2.3
18	Light mountain loam.........	−7.8	−2.0	1.1	3.6	3.4	5.3	9.5	14.2	1.5	2.4
19	Loose light mountain loam.....	−2.3	−1.4	−1.7	−2.1	0.8	7.6	8.0	3.9	1.1	0.3
20	Fine sand.......	4.3	3.4	3.9	3.8	8.9	11.5	9.6	9.8	7.1	6.2
21	Organic loam....	10.1	10.0	9.5	12.6	6.2	−0.7	16.4	16.1	10.9	7.0
22	White clay loam.	−3.5	−3.7	−0.4	−1.4	0.4	−1.0	−2.2	−2.8	−0.3	−0.7

with a high content. It is interesting to note that the mere changing of the water content of a soil changes it from one which is gaining nitrogen to one which is losing. This loss usually occurs under aerobic and not anaerobic conditions as is usually considered requisite for denitrification. The question naturally arises—Can it be associated with the rapid burning out of the organic matter of the soil which is so characteristic of some arid soils? This being the case the question arises as to the specific organisms which bring about the change.

Most soils show a maximum fixation for a specific water content. This, however, varies widely with the soil. In many cases the two maxima appear, —the one when the water content is from 40 to 60 per cent, the other when it is 70 to 100 per cent. This is undoubtedly due to the two groups of organisms—in the first case to the aerobes and in the second to the anaerobes. The average for all soils shows a maximum nitrogen fixation when the soils contain 70 per cent of their water-holding capacity. The average results, considering that containing 70 per cent of water as 100 per cent, are shown graphically in figure 3.
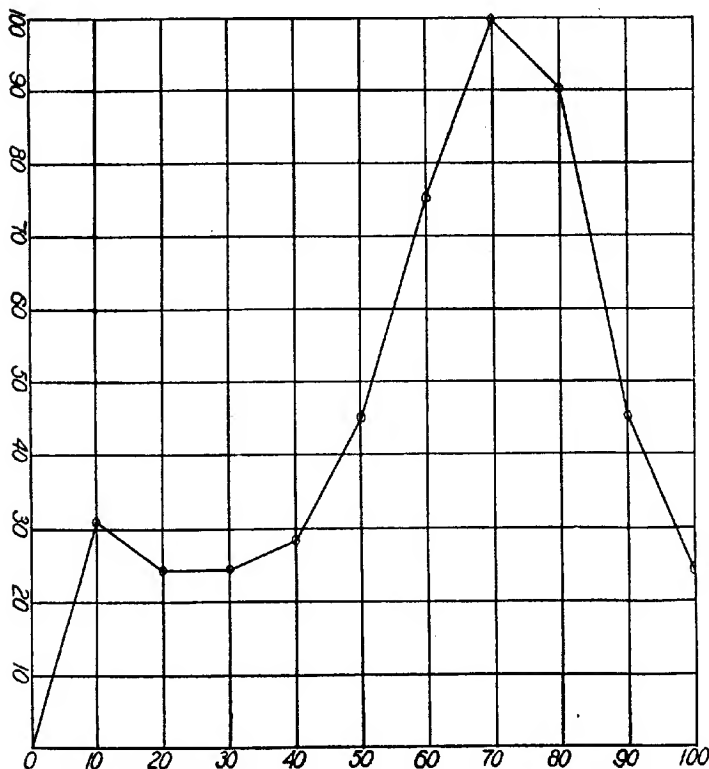
FIG. 3. AVERAGE PERCENTAGE OF NITROGEN FIXED BY SOILS RECEIVING VARYING QUANTITIES OF WATER

The quantity fixed at 70 per cent is taken as 100; on the ordinate is given the per cent of nitrogen fixed, whereas on the abscissa is given water applied as per cent of water-holding capacity

In the main, most soils show a low fixation at the first three water contents and this averages practically the same with 10, 20, 30 and 40 per cent of the water-holding capacity. A few soils fix fair quantities of nitrogen with only 10 per cent of water. The fixation at 90 and 100 per cent is uniformly low, but the very few that show a loss of nitrogen at these water contents would make it appear that water does not favor denitrification in these soils.

RELATIONSHIP BETWEEN AMMONIFICATION, NITRIFICATION, AND NITROGEN FIXATION

The comparative results for ammonification, nitrification, and nitrogen fixation are shown graphically in figure 4.
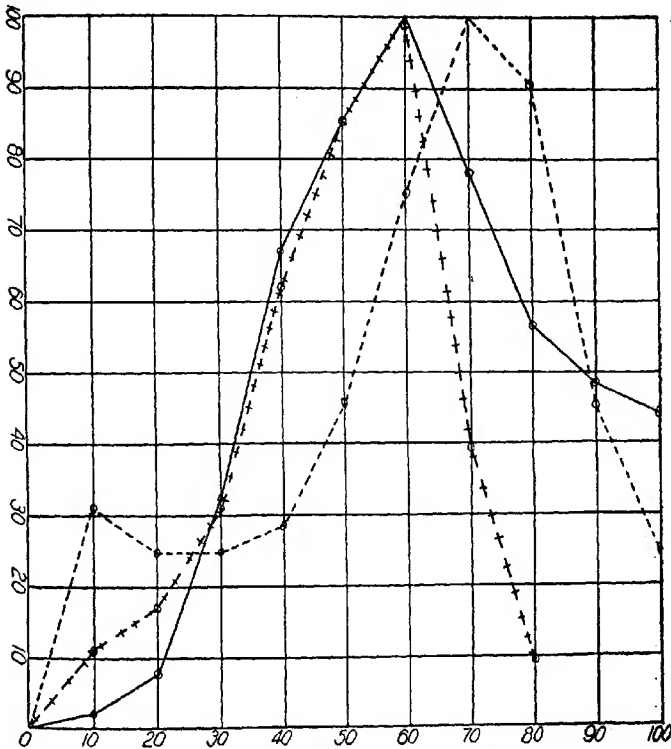
FIG. 4. AVERAGE PERCENTAGES OF AMMONIA ———— AND NITRIC NITROGEN +++ FORMED AND NITROGEN FIXED — — — — IN SOIL RECEIVING VARYING QUANTITIES OF WATER

On the ordinate is given the per cent increase of the respective substances and on the abscissa the quantity of water applied as per cent of water-holding capacity

The point at which maximum activity was obtained has been taken as 100 per cent. Both ammonification and nitrification are at their maximum, according to these results, when the soil contains 60 per cent of the water-holding capacity. However, the individual results showed that it is between 50 and 60 for nitrification and 60 for ammonification.

Only one maximum appears in the average results for nitrogen fixation. This is at 70 per cent and probably favors anaerobic in place of aerobic bacterial activity.

The nitrogen-fixing bacteria do not respond in activity to different water treatments as readily as do the other bacteria. This is probably due to the heterogenous group of organisms which bring about this change in counter-distinction to the homogenous group bringing about nitrification. Eighty per cent of the water-holding capacity reduces nitrogen fixation to 90.4 per cent, ammonification to 56.1 per cent, and nitrification to 9.6 per cent.

BACTERIAL ACTIVITY VS. CROP PRODUCTION

The optimum moisture content for nitrification is not far from the optimum for field crops in general. Wollny (52) placed this at from 60 to 80 per cent of the water-holding capacity of the soil. Mayer (35) placed the opti-

TABLE 7

Water-holding capacity of soils at which maximum crops were obtained

INVESTIGATOR	CROP	PER CENT OF WATER-HOLDING CAPACITY AT WHICH MAXIMUM YIELD WAS OBTAINED
Harris and Maughan (23)	Wheat	60
Harris (22)	Alfalfa	60
Fitthogen (12)	Oats	60–80
Hellriegel (24)	Barley	60
Schroeder	Barley	40–60
Daszenski (5)	Potatoes	Better at 58 than at 33
von Sellhorst (43)	Oats	64–74
Kiesselbach (26)	Corn	60
Maercker (34)	White mustard	60
Wilms (51)	Potatoes	Better at 80 than at 58 or 33
Ohlmer (39)	Wheat	Better at 70 than at 45

mum moisture content of wheat at 80 per cent of the water-holding capacity of the soil, rye at 75 per cent, barley at 75 per cent, and oats at 85 to 90 per cent.

However, the majority of investigators place the optimum moisture for maximum production with most plants at from 60 to 70 per cent, as may be seen from the summarized results in table 7.

The different methods used by the several investigators would account in a measure for the variation in the results reported. Those by Harris, how-

ever, were obtained by the same method as was used in the bacteriological investigations. These, taken in connection with those of the other investigators, indicate that there is a close correlation between the metabolism of the beneficial bacteria and of the higher plants in so far as their water requirements are concerned.

OTHER SOIL CONSTANTS AND THEIR RELATIONSHIP TO BACTERIAL ACTIVITIES

The moisture equivalents of the various soils were determined by the method of Briggs and McLane (1). These, together with the per cent of water necessary for maximum ammonification, are given in table 8.

TABLE 8

Moisture equivalent of soils and per cent of moisture for maximum ammonification

SAMPLE NUMBER	DESCRIPTION	MOISTURE EQUIVALENT	HYDROSCOPIC COEFFICIENT	PER CENT MOISTURE FOR AMMONIFICATION	a_1	a_2
17	Sand	3.32	0.39	18.6	1.807	15.40
20	Fine sand	5.08	1.15	19.8	1.417	6.26
15	Light sandy loam	11.38	3.61	26.4	1.213	3.82
18	Light mountain loam	20.46	4.70	30.6	0.880	3.83
13	Trenton fine loam	21.75	3.61	30.6	0.828	4.99
5	Light sand loam	23.12		31.8	0.830	
11	Silt loam	25.54	4.82	33.6	0.822	4.36
19	Loose light mountain loam	18.32	4.91	33.6	1.146	4.28
6	Clay loam	29.18		34.2	0.740	
9	Black loam	31.47	6.06	35.4	0.725	3.76
16	Medium sand loam	29.36	5.92	36.0	0.797	3.95
10	Very tight clay	39.02	5.57	36.6	0.615	4.32
12	Extra tight clay	45.15	7.77	36.6	0.532	3.09
2	Clay loam	20.65	3.78	36.6	1.162	6.35
4	Sand loam	28.14	4.39	37.2	0.874	5.64
3	Tight clay loam	28.33	4.42	39.0	0.932	5.97
1	Clay loam	23.35	4.24	40.8	1.208	6.65
7	Peaty loam	32.41	6.55	44.4	0.981	4.86
8	Silt loam	30.78	6.66	46.2	1.092	5.04
14	Fine silt loam	35.45	7.83	46.8	0.965	4.37
21	Organic loam	34.02	9.94	46.8	1.005	3.44
22	White clay loam	27.20	8.00	46.8	1.257	4.28

According to Briggs the moisture-holding capacity, the wilting coefficient, the moisture equivalent and the hygroscopic coefficient are related by linear equations thus:

$$c = 2.9\ w + 21.$$
$$c = 1.57\,e + 21.$$
$$c = 4.26\,h + 21.$$

Where c is written for the moisture capacity as defined by Hilgard, w for wilting coefficient, e for moisture equivalent, and h for hygroscopic coefficient.

If therefore the optimum moisture for maximum bacterial activity is directionally proportional to c (in the case of the ammonifiers $c = 0.6$) then these other soil constants may also be related to these constants by a similar set of linear equations.

$$M_a = a_1 e + 12.6, \text{ whence } a_1 = \frac{M_a - 12.6}{e}$$

$$M_a = a_2 w + 12.6, \text{ whence } a_2 = \frac{M_a - 12.6}{w}$$

$$M_a = a_3 h + 12.6, \text{ whence } a_3 = \frac{M_a - 12.6}{h}$$

Writing M_a for the per cent of water for maximum ammonification and a_1, a_2, and a_3 constants, calculated these become $a_1 = 0.942$, $a_2 = 1.74$ and $a_3 = 2.555$; that is the moisture requirement for maximum ammonification may be obtained from any of the soil constants by the following equations:

$$M_a = 0.6\,c$$
$$M_a = 0.942\,e + 12.6$$
$$M_a = 1.74\,w + 12.6$$
$$M_a = 2.55\,h + 12.6$$

a_1 as given in table 8 and calculated from the moisture equivalent, varies from 0.615 to 1.807 with a mean of 0.992. The irregular variation of a_1, as determined from the moisture equivalent, makes it appear evident that the relationship between the moisture equivalent and moisture requirements for maximum ammonification is not as well defined as is the relationship between water-holding capacity and water requirements for maximum ammonification.

The value of a_3 as calculated from the determined hygroscopic moisture varies from 3.09 to 15.4 with a mean of 5.24, thus giving results invariably higher than those obtained by calculating from the Briggs formula.

The relationship between the moisture equivalent and the per cent of moisture for maximum nitrification is shown in table 9.

A set of equations similar to those written for ammonification may be written for nitrification thus, in which the optimum moisture for maximum nitrification is taken at 0.55:

$$M_n = a_1 e + 11.55, \text{ whence } a_1 = \frac{M_n - 11.55}{e}$$

$$M_n = a_2 w + 11.55, \text{ whence } a_2 = \frac{M_n - 11.55}{w}$$

$$M_n = a_3 h + 11.55, \text{ whence } a_3 = \frac{M_n - 11.55}{h}$$

Calculated, these constants become $a_1 = 0.8525$, $a_2 = 1.472$ and $a_3 = 2.163$. Therefore, the moisture requirements for maximum nitrification may be obtained from any of the soil constants by the following equations:

$$M_n = 0.55\,c$$

$$M_n = 0.8525\,e + 11.55$$

$$M_n = 1.472\,w + 11.55$$

$$M_n = 2.163\,h + 11.55$$

a_1 as determined from the moisture equivalent varies from 0.396 to 1.208 with a mean of 0.806. This shows a much wider variation from the moisture equivalent than from the maximum water-holding capacity.

TABLE 9

Moisture equivalent of soils and per cent of moisture for maximum nitrification

SAMPLE NUMBER	DESCRIPTION	MOISTURE EQUIVALENT	PER CENT OF MOISTURE FOR MAXIMUM NITRIFICATION	a_1
17	Sand....................................	3.32	15.5	0.873
20	Fine sand................................	5.08	19.8	1.023
19	Loose light mountain loam.................	18.32	33.6	1.147
15	Light sandy loam.........................	11.38	22.0	0.826
18	Light mountain loam......................	20.46	25.5	0.630
13	Trenton fine loam........................	21.75	25.5	0.593
5	Light sand loam..........................	23.12	26.5	0.601
11	Silt loam................................	25.54	33.6	0.822
6	Clay loam...............................	29.18	34.2	0.749
9	Black loam..............................	31.47	35.4	0.725
16	Medium sand loam........................	29.36	36.0	0.797
10	Very tight clay..........................	39.02	36.6	0.615
12	Extra tight clay.........................	45.15	30.5	0.396
2	Clay loam...............................	20.65	30.5	0.867
4	Sand loam...............................	28.14	37.2	0.876
3	Tight clay loam..........................	28.33	32.5	0.702
1	Clay loam...............................	23.35	40.8	1.208
7	Peaty loam..............................	32.41	44.4	0.981
8	Silt loam................................	30.78	38.5	0.965
14	Fine silt loam...........................	35.45	46.8	0.965
21	Organic loam............................	34.02	46.8	1.005
22	White clay loam.........................	37.20	31.2	0.500

Similar equations may be written for nitrogen fixation in which we find the value of $a_1 = 1.049$, $a_2 = 1.947$ and $a_3 = 2.848$. That is, the moisture requirements for maximum azofication may be obtained from any of the soil constants by the following equations:

$$M_{az} = 0.70\, c$$
$$M_{az} = 1.049\, e + 14.7$$
$$M_{az} = 1.947\, w + 14.7$$
$$M_{az} = 2.848\, h + 14.7$$

SUMMARY

The influence of water upon the bacterial activities of 22 soils were studied. They represent the typical farming lands of Cache Valley—dry land, irrigated, manured and unmanured. They range all the way from a loose sand to a very tight clay and from soils nearly devoid of organic matter to others very rich in organic material. Their moisture-holding capacity varied from 31 to 78 per cent and was closely correlated with the quantity of clay and organic material. Their moisture equivalent varied from 3.32 to 45.15 and the wilting coefficient, as calculated from the moisture equivalent, from 1.80 to 24.54.

Every soil gave a maximum ammonification when it contained 60 per cent of its water-holding capacity of water. Nitrification was at its maximum at 50 or 60 per cent and varied with specific soils. Many of the soils showed two maxima for nitrogen fixation—one at from 50 to 60 and the other from 70 to 80.

The average comparative results for ammonification, nitrification, and nitrogen fixation were as follows:

	PER CENT OF MOISTURE									
	10	20	30	40	50	60	70	80	90	100
Ammonification...........	1.5	8.5	32.4	66.9	81.3	100.0	78.1	56.1	48.5	44.0
Nitrification..............	11.0	16.9	30.7	61.9	85.9	100.0	36.6	9.6		
Nitrogen fixation..........	31.5	24.7	24.7	27.4	45.2	75.3	100.0	90.4	45.2	24.7

Using the formula of Briggs for the moisture equivalent, and the wilting and hygroscopic coefficients, we may write the following equations as representing approximately the water requirements for maximum bacterial activity, where c is written for the moisture capacity as defined by Hilgard, w for the wilting coefficient, e for the moisture equivalent, and h for the hygroscopic coefficient:

$$M_a = 0.6\, c$$
$$M_a = 0.942\, e + 12.6$$
$$M_a = 1.74\, w + 12.6$$
$$M_a = 2.55\, h + 12.6$$
$$M_n = 0.55\, c$$
$$M_n = 0.8525\, e + 11.55$$

$$M_n = 1.472\, w + 11.55$$

$$M_n = 2.163\, h + 11.55$$

$$M_{az} = 0.7\, c$$

$$M_{az} = 1.049\, e + 14.7$$

$$M_{az} = 1.947\, w + 14.7$$

$$M_{az} = 2.848\, h + 14.7$$

REFERENCES

(1) BRIGGS, LYMAN J., AND McLANE, JOHN W. 1907 Moisture equivalents of soils. U. S. Dept. Agr. Bur. Soils Bul. 45.

(2) BRIGGS, LYMAN J., AND SHANTZ, H. I. 1912 The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230.

(3) COLEMAN, L. C. 1908 Untersuchungen über Nitrifikation. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 20, p. 401–420, 484–513.

(4) DAFERT, F. W., AND BOLLIGER, R. 1896 Conservation of farmyard manure. *In* Jour. Chem. Soc. (London), v. 72, pt. 2, p. 590.

(5) DASZEWSKI, A. VON 1900 Der Einfluss des Wasser und der Düngung auf die Zusammensetzung der Asche der Kartoffelpflanze. *In* Jour. Landw., Bd. 48, Heft 3, p. 223–249.

(6) DEHERAIN, P. P. 1887 Sur la production des nitrates dans la terre arable. *In* Ann. Agron., t. 13, p. 241–261.

(7) DEHERAIN, P. P. 1896 Novelle contribution á l'étude de la jachere. *In* Ann. Agron., t. 22, no. 11, p. 515–523.

(8) DEHERAIN, P. P. 1897 Sur la fixation et la nitrification de l'azote dans les terres arables. *In* Compt. Rend. Acad. Sci. (Paris), t. 125, p. 278–283.

(9) DEHERAIN, P. P., AND DEMOUSSY, E. 1896 Sur l'oxydation de la matière organique du sol. *In* Compt. Rend. Acad. Sci. (Paris), t. 123, no. 5, p. 278–282.

(10) ENGBERDING, DIEDRICH 1909 Vergleichende Untersuchungen über die Bakterienzahl im Akerboden in Ihrer Abhangigkeit von Ausseren Einflüssen. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 23, p. 569–642.

(11) FISCHER, HERMANN 1916 The nitrogen-fixing bacteria in water and the soil beneath the water. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 46, p. 304–328. *Abs. in* Exp. Sta. Rec., v. 38, p. 419.

(12) FITTBOGEN, J. 1873 Untersuchungen über das für eine normale Produktion der Haferpflanze nothwendige minimum von Bodenfluchtigkeit, so wie über die Aufnahme von Bestandtheilen des Bodens bei verschiedenem Wassergehalt desselben. *In* Landw. Jahrb., Bd. 2, p. 353–371.

(13) FRAPS, G. S. 1904 Studies in Nitrification. N. C. Agr. Exp. Sta. 26th Ann. Rpt. (1902–03), p. 33–56.

(14) FRAPS, G. S. 1908 The production of active nitrogen in the soil. Texas Agr. Exp. Sta. Bul. 106.

(15) GAINEY, P. L. 1916 The effect of time and depth of cultivating a wheat seed-bed upon bacterial activity in the soil. *In* Soil Sci., v. 2, p. 193–204.

(16) GILTNER, W., AND LANGWORTH, H. V. 1916 Some factors influencing the longevity of soil microörganisms subjected to desiccation with special reference to soil solution. *In* Jour. Agr. Res., v. 5, p. 927–942.

(17) GREAVES, J. E., AND CARTER, E. G. 1916 Influence of barnyard manure and water upon the bacterial activities of the soil. *In* Jour. Agr. Res., v. 6, p. 889–926.

(18) GREAVES, J. E., AND HIRST, C. T. 1917 Some factors influencing the quantitative determination of nitric nitrogen in the soil. *In* Soil Sci., v. 4, no. 3, p. 179–203.

(19) GREAVES, J. E., STEWART, ROBT., AND HIRST, C. T. 1917 Nitrous nitrogen in irrigated soils. *In* Soil Sci., v. 3, p. 149–154.

(20) GREAVES, J. E., STEWART, ROBT., AND HIRST, C. T. 1917 Influence of crop, season, and water on the bacterial activities of the soil. *In* Jour. Agr. Res., v. 9, p. 293–341.

(21) Gunning method modified to include the nitrogen of nitrates 1915–16 Methods of Analysis *In* Jour. Assoc. Off. Agr. Chem., v. 1, p. 8.

(22) HARRIS, F. S. Unpublished results.

(23) HARRIS, F. S., AND MAUGHAN, H. J. 1917 The effect of soil moisture content on certain factors in wheat production. Utah Agr. Exp. Sta. Bul. 152.

(24) HELLRIEGEL, F. H. 1883 Verhältnis zwischen Produktion und Verdunstung—Wie viel Wasser verbraucht eine Pflänze wahrend der Erzengung von einem Gram Trochensubstanz durch schnittlich? *In* HELLRIEGEL: "Beitrage zu den Naturwissenschaftlichen Grundlagen des Ackerhaus Braumschweig, p. 662–664.

(25) KEDING, M. 1908 Weitere Untersuchungen über stickstoffhindende Bakterien. *In* Wiss. Meeresuntersuchungen, N. f. 9, Kiel, 1908, Bd. 33, p. 9. *Abs. in* Centbl. Bakt. (etc.), Abt. 2, Bd. 24, p. 468.

(26) KIESSELBACH, T. A. 1910 Transpiration experiments with the corn plant. *In* Nebr. Agr. Exp. Sta. 23d Ann. Rpt. (1909), p. 125–139.

(27) KING, W. E., AND DORYLAND, C. J. T. 1909 The influence of depth of cultivation upon soil bacteria and their activities. Kan. Agr. Exp. Sta. Bul. 161, p. 211–242.

(28) KRAINSKII, A. V. 1908 *Azotobacter chroococcum*: its physiological properties and activity in the soil. *In* Zhur. Opuitn. Agron. (Russ. Jour. Exp. Landw.), v. 9, no. 6, p. 689–749. *Abs. in* Exp. Sta. Rec., v. 22, p. 427.

(29) KRAINSKII, A. V. 1912 Enriching of soil in nitrogen in connection with the life activity of aerobic microörganisms assimilating free nitrogen. *In* Univ. Izv. [Kief], v. 52, no. 4, p. 1–58. *Abs. in* Exp. Sta. Rec., v. 31, p. 721–722.

(30) LIPMAN, C. B., AND BURGESS, P. S. 1915 Studies on nitrogen fixation and *Azotobacter* forms in soils of foreign countries. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 35, p. 481–511.

(31) LIPMAN, C. B., AND SHARP, L. T. 1915 Effect of moisture content of a sandy soil on its nitrogen-fixing powers. *In* Bot. Gaz., v. 59, p. 402.

(32) LIPMAN, J. G., AND BROWN, P. E. 1909 Report of the soil chemist and bacteriologist. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt. (1908), p. 95–147.

(33) LIPMAN, J. G., BROWN, P. E., AND OWEN, I. L. 1911 Experiments on ammonia and nitrate formation in soils. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 30, p. 156–181.

(34) MAERCKER, MAX 1896 Über die Wirkung der Kolisalze auf Sandboden Arbeiten Deutsche. *In* Landw. Gesel., Heft 20, p. 7–30.

(35) MAYER, A. 1898 Über den Einfluss kleinerer oder grosserer Mengen von Wasser auf die Entwickelung eineger Kulturpflanzen. *In* Jour. Landw., Bd. 46, p. 167–184.

(36) McBETH, I. G., AND SMITH, N. R. 1914 The influence of irrigation and crop production on soil nitrification. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 40, p. 24–51.

(37) MOSIER, J. G., AND GUSTAFSON, A. F. 1917 Soil Physics and Management. J. B. Lippincott Co., Philadelphia and London.

(38) MÜNTER, F., AND ROBINSON, W. P. 1913 Über den Einfluss der Boden und des Wassergehalts auf die Stickstoffumsetzungen. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 39, p. 419–440.

(39) OHLMER, W. 1908 Über den Einfluss der Düngung und der Bodenfeuchtigkeit bei gleichem Strandraum auf die Anlage und Ausbildung der Ähre und die Ausbildung der Kolben beim Göttinger begrannten Squarehead Winterweizen. *In* Jour. Landw., Bd. 56, Heft, 2, p. 153–171.

(40) PATTERSON, J. W., AND SCOTT, P. R.   1912   The influence of soil moisture upon nitrification.   *In* Jour. Dept. Agr. Victoria, v. 10, p. 275–282.

(41) ROBSON, W. P.   1913   Über den Einfluss der Boden und des Wassergehaltes auf die Stickstoffumsetzungen.   Inaug. Diss. Univ. Halle, Wittenberg, p. 91.

(42) SCHLOESING, T., JR.   1897   Contribution a l'étude de la nitrification dans les sols.   *In* Compt. Rend. Acad. Sci. (Paris), t. 125, p. 825–827.

(43) SEELHORST, CONRAD VON   1899   Über den Wasserverbranch der Hoferpflanze bei verschiedenem Wassergehalt und bei verschiedener Düngung des Bodens.   *In* Jour. Landw., Bd. 47, Heft 4, p. 369–378.

(44) SHARP, L. T.   1913   Soil investigations.   *In* Nev. Agr. Exp. Sta. Ann. Rpt. (1912), p. 24–38.

(45) STEWART, ROBT., AND GREAVES, J. E.   1912   The production and movement of nitric nitrogen in soil.   *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 34, p. 115–147.

(46) TRAAEN, A. E.   1916   Über den Einfluss der Feuchtigkeit auf die Stickstoff umsetzungen im Erdboden.   *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 45, p. 119–135.

(47) WARINGTON, ROBT.   1892   Nitrification of soils and manures.   U. S. Dept. Agr. Off. Exp. Sta. Bul. 8, p. 1–94.   (Cites, p. 72, statement by Boussingault.)

(48) WARMHOLD, HERMANN   1906   Untersuchungen über die Biologie Stickstoffbindender Bakterien.   *In* Landw. Jahrb., Bd. 35, p. 1–123.

(49) WIDTSOE, JOHN A.   1898   The chemical composition of Utah soils—Cache and Sanpete counties.   Utah. Agr. Exp. Sta. Bul. 52.

(50) WILLIAMS, BRUCE   1916   Some factors influencing nitrogen fixation and nitrification.   *In* Bot. Gaz., v. 62, p. 311.

(51) WILMS, JOHAN   1899   Einfluss des Wassergehalts und Nährstoffreichtums des Bodens auf die Lebensthätigkeit und Ausbildung der Kartoffelpflanze.   *In* Jour. Landw., Bd. 47, Heft 3, p. 251–292.

(52) WOLLNY, E.   1897   Untersuchung über den Einfluss Wachstumsfaktoren auf des Produktionsvermogen der Kulturpflanzen.   *In* Forsch. Geb. Agr. Phys., Bd. 20, p. 53–109.

(53) YODER, P. A.   1904   A new centrifugal soil elutriator.   Utah Agr. Exp. Sta. Bul. 89.

NUTRIENT REQUIREMENT OF THE POTATO PLANT GROWN IN SAND CULTURES TREATED WITH "TYPE I" SOLUTIONS

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INTRODUCTION

Recent experimental work dealing with the fundamental problem of plant nutrition has been directed mainly to such crop plants as wheat, barley, rice, buckwheat and soybeans grown in water and sand cultures. The potato, an entirely different type of plant and one that presents a number of special problems, has received but little attention. Skinner (13) suggested a method for growing potato plants in water cultures, but apparently little has since been done along this line. Johnston (5) called attention to some of the difficulties encountered in such a study and reported some preliminary work on the nutrient requirement of the potato plant. It was found that fairly uniform sprouts could be obtained for water and sand culture studies from tubers planted in a bed of sawdust. These sprouts did not grow well in water cultures although such a medium is ideal chemically. The medium used in the experiments reported in the present paper was pure white quartz sand treated with the nutrient solutions under consideration.

Two series of experiments were undertaken to determine the best proportion of salts necessary to produce the best growth of potato plants and the greatest yield of tubers. Irish Cobbler was the variety of potato used throughout these experiments.

SERIES I

Introductory

The abnormal growth of the potato plant in water cultures made it impractical to carry on any extensive salt requirement studies where such a medium was used. Sand cultures were therefore selected for these experiments. There are, however, several objections to sand culture methods as often practiced. A change in the moisture content of the sand takes place during the interval between solution renewals. A constant renewal of solutions would overcome this objection, but such a practice is not feasible. There is also a difference in the moisture content of the sand between the beginning and the end of the experiment, even when the cultures are daily brought back to their original weight by the addition of water. As the plant grows, a part of the

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original total weight (mostly water) of the culture is transferred from the sand to the plant. Each time the culture is brought back to its original weight, the total weight is of course the same as the original total weight, but the sand mass is lighter and the plant heavier. With each successive operation the moisture content of the sand becomes less where the plant gains in weight. The weight of the moisture lost from the sand and not the amount lost from the entire culture (plant and sand mass) is the quantity to be added at each renewal. This error is worth noting where plants are used whose weight increases greatly in proportion to the weight of the solution in the sand mass. Other changes are brought about by the selective absorption of elements and ions. With these limitations in mind the sand culture method was employed as best suited to the conditions of the present study.

Procedure and method of experimentation

Potato tubers (4-oz. size) with sprouts just beginning to develop were selected from a lot of home-grown Irish Cobblers and planted 3 to 4 inches deep in sawdust in one of the greenhouses of the Maryland Agricultural Experiment Station on October 8, 1919. About 7 weeks later (November 25) the sprouts were separated from their tubers, washed in tap water and divided into three groups according to size and development. Group A was composed of sprouts with 6 to 8 leaves well developed, but not of full size, group B was composed of sprouts with 4 or 5 leaves well started and group C of sprouts with 1 or 2 leaves started or with leaves just beginning to open from their buds. These sprouts were then washed in distilled water.

Three sprouts with well developed roots, one from each group, were weighed and placed in a 1-gallon glazed earthenware jar containing 4500 gm. of air-dry sand[1] and 1000 cc. of nutrient solution. The sand was then flooded by adding 500 cc. more of the nutrient solution, thus making the final adjustment of the plants in the sand very easy. Enough of the solution was then drawn off to bring the level of the water-table below the surface of the sand. The following morning more of the solution was withdrawn to reduce the total amount to 675 gm. There were then 675 gm. of solution to 4500 gm. of air-dry sand, or 15 per cent of the dry weight of the sand was the weight of the solution in the culture. A small collar of cotton was placed around the stem of each plant at the surface of the sand and a wax seal (4 parts parawax and 1 part white vaseline by weight) similar to that used by Briggs and Shantz (1) was poured over the surface of the sand at a temperature of 50° to 60°C. The cotton served the double purpose of protecting the plants from the hot wax and providing space for the transverse growth of the stems. Twenty-two cultures were thus prepared, weighed and placed on a rotating table similar to that employed by Shive (11). An atmometer corrected to the Livingston

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[1] The sand used in the experiments of series I was not washed since it was relatively free from impurities.

(6) standard spherical atmometer was operated on the table with the cultures and a maximum-minimum thermometer was suspended in the shade beneath the table.

The method of renewing solutions differed somewhat from those employed by McCall (7), McCall and Richards (8) and Shive and Martin (12). Each pot was provided with a glass tube, of about 4-mm. bore, extending to the bottom where it made a 90° angle and ended in a funnel-shaped opening.
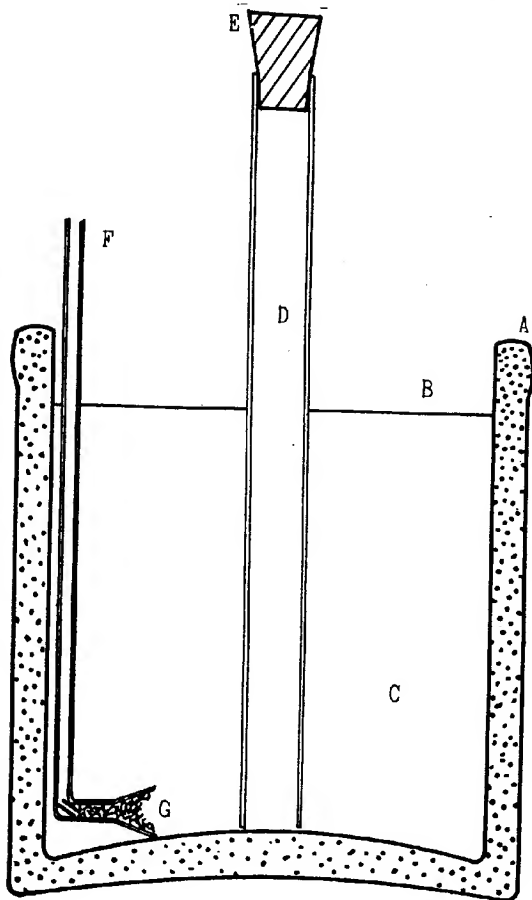
FIG. 1. DIAGRAM SHOWING CROSS-SECTION OF CULTURE POT AND TUBES FOR RENEWING AND FOR WITHDRAWING SOLUTIONS

A, glazed earthenware pot; B, wax seal; C, sand mass; D, supply tube with cork stopper E; F, outlet tube for withdrawing solutions by suction; G, glass wool.

A small glass tube was inserted from this larger opening into the bend of the tube and a tuft of glass wool wedged in next to the small piece of tubing. Suction was applied at the upper end of this tube whenever the solution was drawn off. Another glass tube, 2 cm. in diameter, ran through the center of the sand mass to the bottom of the pot. Into this tube fresh solutions were poured. These tubes are diagrammatically represented in figure 1. Solutions added at the bottom and allowed to rise through the sand are likely to disturb the plant roots less than those added at the top that flow rapidly down over

TABLE 1

Molecular proportions and partial volume-molecular concentrations of monobasic potassium phosphate, calcium nitrate and magnesium sulfate required to produce 21 solutions, each having a calculated osmotic pressure of 1.00 atmosphere at 25°C.

CULTURE NUMBER	SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME-MOLECULAR CONCENTRATIONS		
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
1	IR_1S_1	1	1	6	0.0027	0.0027	0.0161
2	S_2	1	2	5	0.0025	0.0049	0.0123
3	S_3	1	3	4	0.0024	0.0071	0.0094
4	S_4	1	4	3	0.0022	0.0089	0.0067
5	S_5	1	5	2	0.0022	0.0108	0.0043
6	S_6	1	6	1	0.0020	0.0122	0.0020
7	R_2S_1	2	1	5	0.0053	0.0027	0.0132
8	S_2	2	2	4	0.0049	0.0049	0.0099
9	S_3	2	3	3	0.0047	0.0071	0.0071
10	S_4	2	4	2	0.0045	0.0090	0.0045
11	S_5	2	5	1	0.0041	0.0104	0.0021
12	R_3S_1	3	1	4	0.0076	0.0025	0.0101
13	S_2	3	2	3	0.0072	0.0048	0.0072
14	S_3	3	3	2	0.0068	0.0068	0.0045
15	S_4	3	4	1	0.0065	0.0086	0.0021
16	R_4S_1	4	1	3	0.0099	0.0025	0.0074
17	S_2	4	2	2	0.0094	0.0047	0.0047
18	S_3	4	3	1	0.0090	0.0068	0.0022
19	R_5S_1	5	1	2	0.0123	0.0024	0.0049
20	S_2	5	2	1	0.0118	0.0047	0.0023
21	R_6S_1	6	1	1	0.0145	0.0024	0.0024

the roots. At the time the solutions were renewed distilled water was first added to bring the total weight up to the original total weight. Enough solution (500 cc.) was then added to bring the level of the water-table to the surface of the sand. After standing about four minutes suction was applied to the smaller tube and the culture brought back to its original weight.

The solutions used were those designated as type I by the Committee on Salt Requirements of Representative Agricultural Plants.[2] These solutions

[2] See specially prepared plans on the salt requirements of representative agricultural plants to be obtained from the chairman of Committee on Salt Requirements of Representative Agricultural Plants, Laboratory of Plant Physiology, Johns Hopkins University, Baltimore, Maryland.

were composed of 21 combinations of the three salts; monobasic potassium phosphate (KH_2PO_4), calcium nitrate ($Ca(NO_3)_2$) and magnesium sulfate ($MgSO_4$) when the partial osmotic pressure of each varied by equal increments of one-eighth of the total osmotic pressure. The initial total osmotic pressure of each solution was approximately 1.00 atmosphere. The molecular proportion and the partial volume-molecular concentration of each salt in each of the 21 solutions is given in table 1. An additional culture treated with distilled water was introduced into the series for comparison. No ferric phosphate was added to these cultures as there was a sufficient amount of iron in the sand for plant use. With but few exceptions, solutions were renewed twice a week over a period of 8 weeks. Where these exceptions occurred distilled water was added to bring the cultures back to their original weights.

Data on transpiration, evaporation and temperature were obtained at the time the solutions were renewed. At the conclusion of the experiment, data on the relative vigor and appearance of the plants, stem height, green weight of the plants and of the tubers and dry weight of the plants were obtained. Only such data are here presented as bear on the problem of selecting the best proportions of these three salts for good growth of the potato plant and its tubers.

Presentation of results

There was considerable variation in the general vigor or health of plants in the same cultures. This variation was probably due in part to the fact that sprouts of three different sizes were planted in each pot and to the use of home-grown seed of this particular variety which is not nearly so uniform as that grown at higher altitudes or farther north. General vigor or health of individual plants can not very well be measured quantitatively, so a method of scoring as described by Free (2) was used to indicate this quality of the plants. Each plant was compared only with the plants of its particular group, as for example, all plants in group A were compared with each other. An illustration of the variation in behavior of sprouts of the three sizes when grown in the same medium is shown in culture 5. Two plants (A and C) of this culture have the highest numerical score, but plant B has a score next to the lowest. These data, with those showing the approximate percentages of yellow leaf area, are presented in table 2. The greatest percentage of yellow leaf area occurs in culture 22 where nothing but distilled water was used.

The total green weight of the sprouts just before planting and the total green weight of plants (tops and roots without new tubers) at the time of harvest are given per culture in table 3 in grams and in numbers relative to the average total weight of sprouts. These sprouts when planted were not as uniform as was desired, but an examination of the ratios of final to original green weight shows the effect of various cultural treatments to be greater than that expected from individual variation alone. The final green weight

of plants is greatest for culture 15 while that of culture 14 is second. Cultures 5, 8 and 9 also are very good. The greatest ratio value occurs for culture 20, but this is due to the low initial weight of sprouts. The green weights of new tubers produced by the plants of this series are given in the last two columns of table 3. The seven cultures producing the greatest weight of tubers are 5, 14, 4, 6, 15, 13 and 8. The average weight per culture of the entire series is 32.3 gm.

TABLE 2

Relative vigor and percentage of yellow leaf area in potato plants of series I at the time of harvest

CULTURE NUMBER	NUMERICAL SCORE[*] OF GENERAL VIGOR			APPROXIMATE PERCENTAGE OF YELLOW LEAF AREA[†]		
	A	B	C	A	B	C
1	6	4[b]	10[b]	30	2	4
2	7	15	13[bb]	4	2	1
3	10	3	10	12	5	0
4	18	12	17	6	8	0
5	21	1	21	1	25	0
6	16	9	13[b]	2	70	2
7	11	2	3	25	5	10
8	17	7	19	15	10	0
9	20	16	18	2	2	0
10	8	16[b]	6	7	0	1
11	12	14	10[b]	1	3	0
12	3[a]	7[b]	9	10	10	3
13	18[b]	12[b]	8	7	4	2
14	15	21	14	2	2	6
15	14	19	20	1	10	10
16	1	6	4[b]	25	18	16
17	3[a]	11	11	1	12	0
18	11[b]	20	13	1	10	5
19	5	6	4	8	8	2
20	7[b]	18	1	7	0	0
21	2	4	2	4	40	0
22	0	0	0	100	100	80

[*] In several cases plants have the same numerical score. Where such cases occur the better plant is indicated by the superscript (b) while those that are alike have the superscript (a).

[†] These estimates were made by Prof. J. B. S. Norton.

The data of table 3 are perhaps more clearly presented as graphs in figure 2. The culture numbers are given in order along the abscissa. The first heavy line over the first six culture numbers represents the first row of cultures in the triangle diagram described in various publications[3] and illustrated in figures 4 and 5. The second heavy line over cultures 7 to 11, inclusive, is the second row of cultures in the triangle diagram. The third heavy line is the third row of cultures, etc., culture 21 being the apex of the triangle. All

[3] For descriptions of triangular diagrams and their application to plant nutrition work see Hibbard (3), McCall (7), Shive (11), Schreiner and Skinner (9, 10) and Tottingham (14).

graphs are plotted from the relative numbers in the table. In the lower half of the figure the full horizontal line represents the average green weight of sprouts at the time of planting, the broken line represents the green weight of sprouts of individual cultures and the full irregular line represents the green weight of the plants (tops and roots without tubers) at the time of harvest. Variation in weight of the sprouts is shown by the departure of

TABLE 3

Green weight of potato sprouts when planted and of plants and tubers at harvest with ratio of original to final green weight of plants given per culture of series I

CULTURE NUMBER	TOTAL GREEN WEIGHT OF PLANT				RATIO OF FINAL TO ORIGINAL GREEN WEIGHT	TOTAL GREEN WEIGHT OF TUBERS	
	Original		Final				
	Actual	Relative to average 18.1 gm.	Actual	Relative to average 18.1 gm.		Actual	Relative to average 32.3 gm.
	gm.		*gm.*			*gm.*	
1	17	0.94	33.1	1.83	1.95	25.7	0.80
2	16	0.88	33.5	1.85	2.09	25.0	0.77
3	17	0.94	38.2	2.11	2.25	27.0	0.84
4	21	1.16	49.9	2.76	2.38	39.9	1.24
5	21	1.16	52.6	2.91	2.50	43.6	1.35
6	22	1.22	48.1	2.66	2.19	39.9	1.24
7	26	1.44	42.2	2.33	1.62	29.2	0.90
8	25	1.38	53.9	2.98	2.16	37.4	1.16
9	20	1.10	53.7	2.97	2.68	29.3	0.91
10	16	0.88	44.3	2.45	2.77	31.3	0.97
11	18	0.99	45.4	2.51	2.52	35.4	1.10
12	16	0.88	32.1	1.77	2.01	23.2	0.72
13	18	0.99	50.5	2.79	2.81	38.8	1.20
14	18	0.99	56.7	3.13	3.15	42.2	1.31
15	20	1.10	57.4	3.17	2.87	39.9	1.24
16	17	0.94	45.6	2.52	2.68	28.5	0.88
17	16	0.88	44.3	2.45	2.77	26.7	0.83
18	16	0.88	50.9	2.81	3.18	33.3	1.03
19	16	0.88	38.3	2.12	2.39	32.0	0.99
20	12	0.66	38.6	2.13	3.22	35.4	1.09
21	15	0.83	35.8	1.98	2.39	27.8	0.86
22	17	0.94	22.8	1.26	1.34	18.1	0.56

the broken line from the horizontal line. The production of tops and roots is greatest in culture 15 with culture 14 a close second. In general as the position of a culture is moved from left to right along the heavy lines representing the culture rows the final green weight is increased. This suggests that either an increase in the proportion of calcium nitrate or a decrease in the proportion of magnesium sulfate increases the green weight production. There are, however, indications that a too great proportion of calcium nitrate or a too small proportion of magnesium sulfate decreases green weight pro-

duction. The question as to whether the calcium nitrate or the magnesium sulfate is the controlling salt will be discussed later.

In the upper portion of figure 2 graphs of tuber production (broken line) and of the ratio of final green weight to original green weight of the plants (full line) are shown. There is a striking similarity between the graph of tuber production and that of final green weight production. With but few exceptions the shapes and slopes of the curves are identical. The ratio graph has the appearance of a series of rises with each succeeding rise higher than the one immediately preceding. The highest point (culture 20) as stated
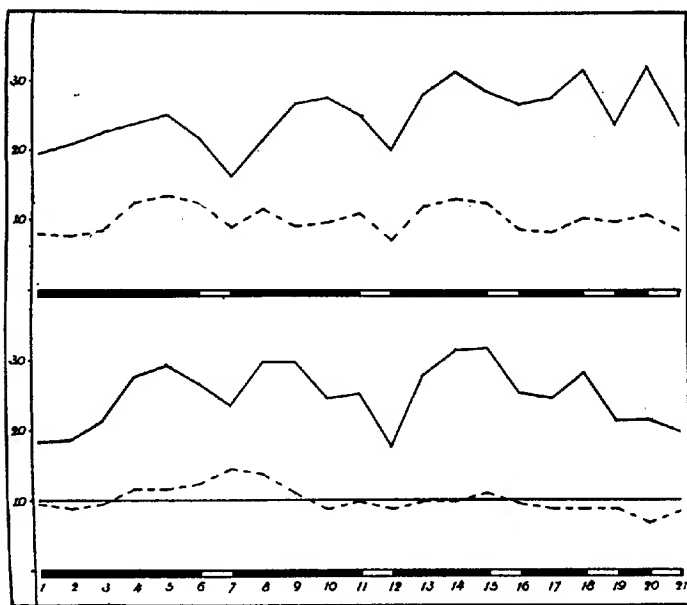
FIG. 2. Graphs of series I showing average green weight of sprouts (horizontal line), green weights of sprouts per culture (broken line) and green weight of plants per culture at harvest (full line) in lower part of figure; green weight of tubers (broken line) and ratio of final green weight of plants to green weights of sprouts (full line) for each culture in upper part of figure.

before is largely due to the low weight of these sprouts at the time of planting. The weight of sprouts in culture 20 was farthest below the average for the series while that of culture 7 was highest above the average, which latter fact accounts for the very low drop in the upper graph for culture 7.

The stem height of each plant (distance from surface of wax seal to base of terminal bud) is recorded in table 4. The variation in stem height between plants of the same culture is considerable in several cases, but when the

averages are compared culture 8 is found to contain the tallest plants and 22 the shortest. All the plants were more or less dwarfed. The transpirational water loss of each culture for the entire period is also given in this table, as well as the dry weights of the plants. Before obtaining the dry weights, the plants were first air-dried in the greenhouse and then placed in an electric vacuum oven at a temperature of 83°C. for 24 hours. The water require-

TABLE 4

Stem height of potato plants and transpirational water loss, dry weight of plants and water requirement per culture of series I

CULTURE NUMBER	STEM HEIGHT				TOTAL TRAN-SPIRATION	DRY WEIGHT OF TOPS AND ROOTS	WATER REQUIREMENT
	Plant a	Plant b	Plant c	Average			
	cm.	*cm.*	*cm.*	*cm.*	*gm.*	*gm.*	
1	7.0	1.0	2.0	3.3	1234	2.9	425
2	3.4	3.7	2.0	3.0	1109	2.8	396
3	6.4	2.2	2.4	3.7	1230	2.6	473
4	10.1	2.5	3.5	5.4	1616	4.0	404
5	11.5	1.2	3.3	5.3	1957	4.1	477
6	9.4	2.0	2.5	4.6	1730	3.7	468
7	5.0	2.1	3.0	3.4	1144	3.2	357
8	9.5	5.6	3.0	6.0	1437	4.5	319
9	8.5	2.4	5.0	5.3	1698	5.0	340
10	8.1	4.1	4.5	5.6	1203	4.2	287
11	10.3	3.8	3.6	5.9	1465	3.3	444
12	4.3	3.9	2.1	3.4	905	2.4	377
13	6.5	3.0	3.0	4.2	1544	3.5	441
14	6.4	6.6	2.5	5.2	1697	3.3	514
15	5.6	4.4	4.4	4.8	1523	4.7	324
16	3.5	1.4	3.3	2.7	890	2.8	318
17	3.3	2.0	3.0	2.8	934	3.6	259
18	6.5	2.5	2.5	3.8	1439	3.9	369
19	3.5	3.2	2.2	3.0	1030	2.4	429
20	3.5	4.4	1.6	3.2	1201	2.6	462
21	2.7	4.0	1.8	2.8	857	2.2	390
22	1.5	1.7	1.5	1.6	565	1.3	435
Average				4.0	1291		396

ments of these plants (transpiration per unit dry weight of plant) are given in the last column of the table. The mean water requirement for the entire series is 396 with a standard deviation of 67 ± 6.8.

SERIES II

Introductory

The culture pots used in series II had a capacity of 2 gallons each instead of 1 gallon as in series I. The amount of sand used was just twice that used in the first series, but the same moisture content (15 per cent based on the

weight of air-dry sand) was maintained. This increase in the size of the culture pot was made in order to give the roots and tubers ample room for growth and to maintain a more uniform moisture content. The amount of water lost by transpiration for a period of three or four days is relatively great for large plants. By increasing the total amount of solution and size of container the percentage of drying out is much less than would be the case where a smaller container is used. The percentage decrease of any one ion or molecule is also much less where greater amounts of solution are used. These points have been emphasized by Hoagland (4) and deserve more attention than has heretofore been given them.

Procedure and method of experimentation

The arrangement of tubes and plants in the pots was the same as that employed in series I. As has been stated, the amount of sand used was twice as great and hence the amount of solution was doubled in order to maintain the same moisture content as the cultures of series I. This weighed amount of air-dry sand was placed in each of 22 pots and then carefully washed with tap water and later with distilled water. The sand was then flooded with the solution to be used in that particular pot.

The seed ends of 144 Western-Maryland-grown Irish Cobbler potatoes were planted in a bed of sawdust on February 12, 1920. On March 18, sprouts similar in size and appearance with four or five well developed leaves were detached from their tubers and washed in tap water and then in distilled water. These sprouts were planted in sand, three to a container, and the cultures treated in a manner similar to that of series I. The check culture, number 22, was treated somewhat differently, however. A seed piece with three sprouts of the size used in the other cultures was carefully selected. This seed piece with its three sprouts was planted in the sand and treated in the same manner as the other 21 cultures with the exception that distilled water was used instead of a nutrient solution.

The weight of the cultures (about 14 or 15 kilos each) made it impracticable to use the rotating table employed in series I. A stone top table in the central part of the greenhouse was therefore used to support these cultures. To facilitate flooding the sand with solutions at the time the solutions were renewed, a liter flask containing the proper solution was placed on a small shelf in front of the culture and connected by a siphon to the central glass tube in the pot. The bottom of the flask was raised slightly higher than the surface of the wax seal. Such an arrangement of siphons made it possible to flood all the cultures at the same time. The cultures were permitted to remain in this saturated condition for several minutes before suction was applied and the total weight of each reduced to its original value.

Presentation of results

The three plants of each culture in the second series were much more uniform in appearance than those of the first series.   No special scoring of relative health or vigor was made, but measurements similar in character to those of the earlier series were recorded during the experiment and at the time of harvest (May 6) and similar tables and graphs constructed.

TABLE 5

Green weight of potato sprouts when planted and of plants and tubers at harvest with ratio of original to final green weight of plants given per culture of series II

| CULTURE NUMBER | TOTAL GREEN WEIGHT OF PLANT | | | | RATIO OF FINAL TO ORIGINAL GREEN WEIGHT | TOTAL GREEN WEIGHT OF TUBERS | |
| | Original | | Final | | | | |
	Actual	Relative to average 25.4 gm.	Actual	Relative to average 25.4 gm.		Actual	Relative to average 81.8 gm.
	gm.		*gm.*			*gm.*	
1	25.0	0.98	54.8	2.16	2.19	48.9	0.60
2	27.6	1.09	77.9	3.07	2.82	65.5	0.80
3	25.4	1.00	94.6	3.72	3.72	87.2	1.07
4	24.8	0.98	90.4	3.56	3.65	78.9	0.96
5	26.7	1.05	107.4	4.23	4.02	91.4	1.12
6	25.0	0.98	93.3	3.67	3.73	81.8	1.00
7	24.7	0.97	52.0	2.05	2.11	48.0	0.59
8	28.1	1.11	83.4	3.28	2.97	77.2	0.94
9	25.1	0.99	102.5	4.04	4.08	91.7	1.12
10	25.3	1.00	120.3	4.74	4.76	132.5	1.62
11	23.1	0.91	109.0	4.29	4.72	105.8	1.29
12	28.4	1.12	53.6	2.11	1.89	49.0	0.60
13	25.5	1.00	98.8	3.89	3.87	91.8	1.12
14	24.3	0.96	128.3	5.05	5.28	113.0	1.38
15	27.4	1.08	160.0	6.30	5.84	127.0	1.55
16	26.5	1.04	66.2	2.61	2.50	51.2	0.63
17	23.1	0.91	85.0	3.35	3.68	89.4	1.09
18	25.3	1.00	124.6	4.91	4.93	130.3	1.59
19	24.4	0.96	50.4	1.98	2.07	39.6	0.48
20	23.3	0.92	81.3	3.20	3.49	80.0	0.98
21	23.6	0.93	49.0	1.93	2.08	37.6	0.46
22	122.5*†		121.4*			49.4†	0.60

* Includes weight of old tuber. These weights are not included in the average weight.

† Weight not included in average weight.

Table 5 gives data of the total green weight of sprouts per culture when planted and that of the plants at the time of harvest, 7 weeks later.   The ratio of final to original green weight and the green weight of tubers produced per culture also are presented in this table.

These data are represented graphically in figure 3, which is constructed from the relative numbers in table 5.   The deviation in the weight of sprouts
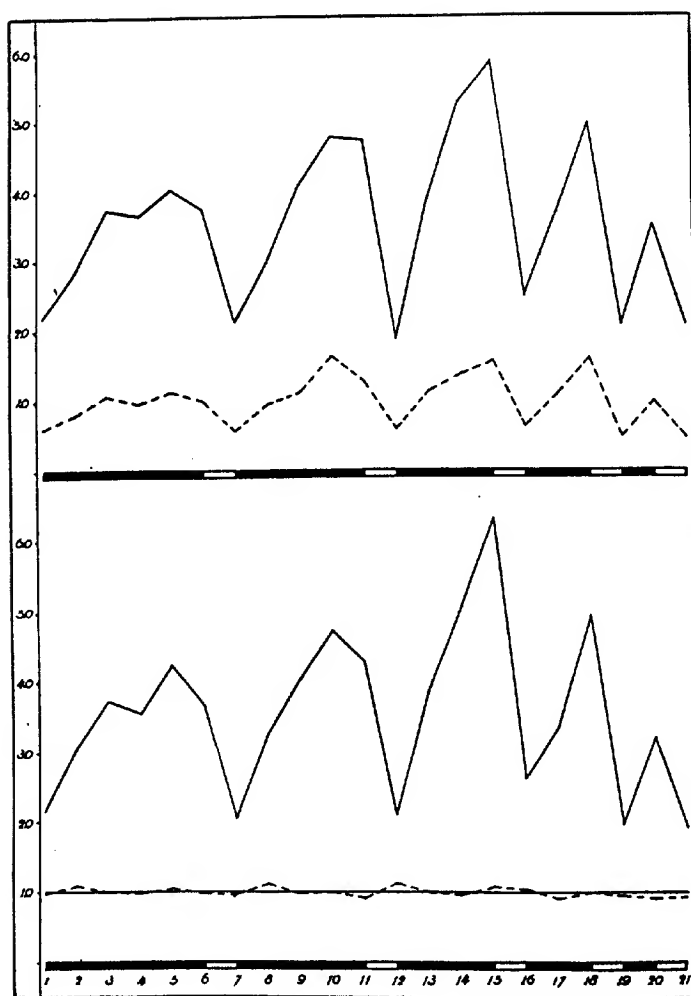
Fig. 3. Graphs of series II showing average green weights of sprouts (horizontal line), green weight of sprouts per culture (broken line) and green weight of plants per culture at harvest (full line), in lower part of figure; green weight of tubers (broken line) and ratio of final green weight of plants to green weights of sprout (full line) for each culture, in upper part of figure.

(lower broken line) from the average of the series (full horizontal line) is seen to be very small while differences in the final weight of plants (lower full line) are very marked.   Culture 15 shows the greatest green weight.   The series of peaks in the graph are located above the culture numbers near the right end of the heavy lines representing the culture rows of the triangle.   The cultures having low green weight values are at the left end of the culture-row lines in every case.   The graph of tuber production (upper broken line) is very similar to that of the final green weight.   The maximum production

TABLE 6

Stem height of potato plants and transpirational water loss, dry weight of plants and water requirement per culture of series II

CULTURE NUMBER	STEM HEIGHT				TOTAL TRAN-SPIRATION	DRY WEIGHT OF TOPS AND ROOTS	WATER REQUIREMENT
	Plant a	Plant b	Plant c	Average			
	cm.	*cm.*	*cm.*	*cm.*	*gm.*	*gm.*	
1	6.8	7.0	5.5	6.4	2175	5.2	418
2	11.5	12.0	11.4	11.6	3127	8.1	386
3	12.9	11.4	14.0	12.8	4018	10.4	386
4	14.8	13.5	12.1	13.5	3590	12.1	297
5	11.0	15.5	17.0	14.5	4139	9.8	422
6	11.8	14.0	16.9	14.2	3813	9.3	410
7	4.5	5.3	5.5	5.1	1970	5.0	394
8	9.6	10.5	10.0	10.0	3287	7.7	427
9	13.8	15.7	14.5	14.7	4120	10.0	412
10	15.3	20.3	19.7	18.4	5138	14.2	362
11	16.8	15.9	17.5	16.7	4540	11.1	409
12	4.5	5.5	5.0	5.0	2272	5.2	437
13	10.7	10.7	9.5	10.3	3974	9.5	418
14	16.4	18.7	17.8	17.6	5066	13.1	387
15	12.7	22.8	20.0	18.5	5643	17.5	322
16	6.6	5.8	5.5	6.0	2303	5.7	404
17	11.3	12.2	7.5	10.3	3456	7.8	443
18	16.0	19.7	14.7	16.8	5163	11.3	457
19	4.4	5.9	8.0	6.1	1812	4.0	453
20	7.4	10.0	10.1	9.2	3146	7.1	443
21	5.0	4.0	6.7	5.2	1762	4.1	430
22	5.1	6.4	3.1	4.9	1122	2.3	488
Average				11.3	3438		409

occurs in culture 10 rather than in culture 15, however.   Very little difference is noticed between cultures 10, 15 and 18 in tuber yield.   The ratio graph (upper full line) showing the gain in green weight of the plants has its maximum at culture 15 and its minimum at 12.   The two preceding and the two following maxima decrease in value the farther they are removed from this central maximum.   With the shortening of the heavy base lines representing culture rows the proportion of potassium phosphate increases.   The series of maxima in the upper graph indicates that with an increase of potassium

phosphate up to the third row, better plants are produced, but beyond that row decreased growth is noticed. The graph also shows that an increase of calcium nitrate up to the fourth or fifth culture of each row, where the rows are that long, produces increased growth. This is indicated by the rise in the curve above the heavy base lines representing culture rows.

Measurements of stem height per plant and the average for each culture are presented in table 6. Transpirational water loss for the entire period, dry weight of plants and the water requirement also are given for each culture in this table. The maximum average height value occurs for culture 15 and the minimum value for culture 22. These same cultures have the maximum and minimum transpirational values, respectively. The dry weight value for culture 15 is almost eight times that of the minimum, 2.3 gm. for culture 22. Since culture 22 received no fertilizer treatment and its plants were attached to their seed piece it is not comparable with any of the 21 cultures of the triangle. The maximum average height value of culture 15 is almost four times that of culture 12 of the triangle and its dry weight value is more than four times that of culture 19, the two cultures whose respective values are lowest. No such variations between cultures occur for the water requirement. The mean water requirement for this series is 409 with a standard deviation of 42 ± 4.3.

CONCLUSIONS

Introductory

Potato plants of corresponding cultures of the two series show great similarity in their reaction to the same salt proportions of the 21 different treatments. There are minor variations, but these are to be expected where a plant of considerable individual variation is employed, especially when two lots of seed are used. There is also the possibility of seasonal conditions bringing about different kinds of reactions in plants at different stages of development. This may account for the variation between plants of the same cultures in series I for the sprouts in each culture of this series were of three different sizes when planted. The seasonal differences, together with the use of home-grown seed, no doubt account for the smaller plants produced in series I. This series was grown at a time of year when light conditions were at a minimum. In spite of minor differences between the two series the results can be legitimately averaged and general conclusions deduced therefrom.

The green weight of the plants at the time of harvest, the dry weight of these same plants, the green weight of tubers and the water requirement for corresponding cultures of these two series have been averaged and are presented in table 7. All the average weights are given in grams per culture and as numbers relative to the average of each respective kind of measurement. The water requirement values are the averages of those given in tables 4 and 5.

TABLE 7

Average green and dry weights of potato plants, average green weight of tubers and average water requirement for corresponding cultures of series I and II

CULTURE NUMBER	WEIGHT OF PLANTS (TOPS AND ROOTS)				GREEN WEIGHT OF TUBERS		WATER REQUIREMENT
	Green weight		Dry weight				
	Actual	Relative to average 67.4 gm.	Actual	Relative to average 6.2 gm.	Actual	Relative to average 57.4 gm.	
	gm.		gm.		gm.		
1	44.0	0.65	4.1	0.66	37.3	0.65	422
2	55.7	0.83	5.5	0.89	45.3	0.79	391
3	66.4	0.99	6.5	1.05	57.1	0.99	430
4	70.2	1.04	8.1	1.31	59.4	1.03	351
5	80.0	1.19	7.0	1.13	67.5	1.18	450
6	70.7	1.05	6.5	1.05	60.9	1.06	439
7	47.1	0.70	4.1	0.66	38.6	0.67	376
8	68.7	1.02	6.1	0.98	57.3	1.00	373
9	78.1	1.16	7.5	1.21	60.5	1.05	376
10	82.3	1.22	9.2	1.48	81.9	1.43	325
11	77.2	1.15	7.2	1.16	70.6	1.23	427
12	42.9	0.64	3.8	0.61	36.1	0.63	407
13	74.7	1.11	6.5	1.05	65.3	1.14	430
14	92.5	1.37	8.2	1.32	77.6	1.35	451
15	108.7	1.61	11.1	1.79	83.5	1.45	323
16	55.9	0.83	4.3	0.69	39.9	0.70	361
17	64.7	0.96	5.7	0.92	58.1	1.01	351
18	87.8	1.30	7.6	1.23	81.8	1.43	413
19	44.4	0.66	3.2	0.52	35.8	0.62	441
20	60.0	0.89	4.9	0.79	57.7	1.01	453
21	42.4	0.63	3.2	0.52	32.7	0.57	410

Plant production

Green weight. The maximum green weight is 108.7 gm. for culture 15. This represents the weight of three plants. The minimum weight is 42.4 gm. for culture 21. The maximum is approximately two and one-half times greater than the minimum. The average weight for the entire series is 67.4 gm. The weights of the individual cultures are expressed in the third column of the table as numbers relative to this average, for the purpose of simplifying comparisons between cultures. Eleven cultures are above the average, or 1.00, and the remaining ten below. Cultures 3 and 17 are almost as good as the average, however.

The green weights of these plants are represented diagrammatically in figure 4. In general form the figure is similar to those ordinarily used to represent a triangle of cultures. Instead of merely representing the high and low areas, this figure has been made to resemble a contour map. The seven cultures of lowest values are shown in the "swamp land" while the other

fourteen are placed at different "elevations" by contour lines. These contour lines are drawn for every 10 gm. of green weight above 60 gm. The cultures are numbered in regular order in the triangle. Culture 17, for example, lies between the contour lines 60 and 70. The plants of this culture have a total green weight between 60 and 70 gm. Cultures 3, 8 and 20 lie between the same contour lines. Culture 15 has the highest value and is encircled by a
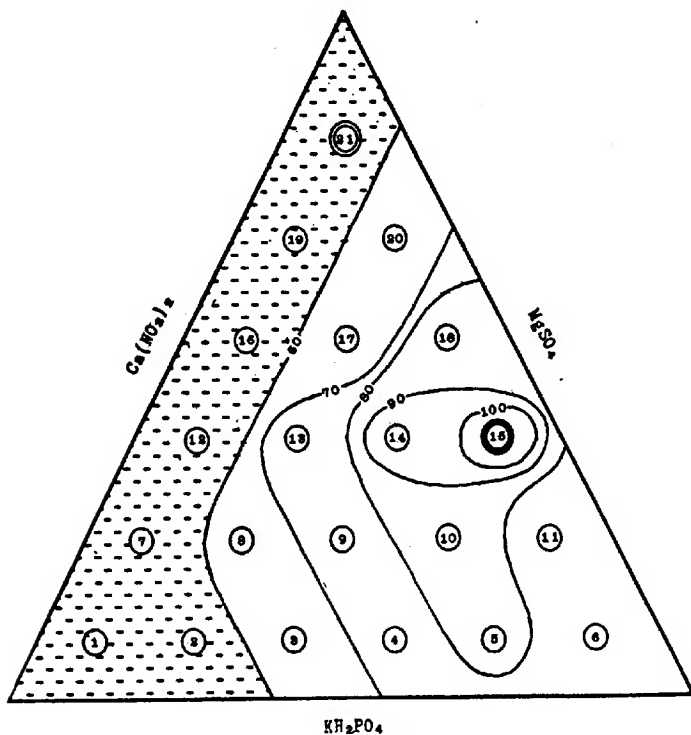
FIG. 4. DIAGRAM SHOWING THE APPROXIMATE GREEN WEIGHTS OF POTATO PLANTS (TOPS AND ROOTS) PER CULTURE

The seven lowest-yielding cultures are within the shaded area below the 60-gm. contour line; the culture giving the highest yield is marked by the heavy circle, the lowest by the double circle.

heavy ring. Culture 21 has the lowest value and is encircled by a double ring. Such a diagram enables one to see at a glance the relation of various cultures to each other with respect to their comparative yields and to their position in the triangle, thus showing their relation to various proportions of the three salts.

When the green weight is used as a criterion, greatest growth is obtained from cultures high in calcium nitrate, low in magnesium sulfate and with a medium amount of potassium phosphate. The question previously raised as to whether high calcium nitrate or low magnesium sulfate is responsible for this better growth can be answered in part. Further experimentation, however, where the ions and elements of these salts are interchanged and are used in other combinations, must be carried out before the controlling ions and elements of these salts can be definitely known in their true relation to the growth and development of the plant. An examination of plate I showing the plants of series II will bring out certain facts. Cultures lying along the calcium nitrate side of the triangle, numbers 1, 7, 12, 16, 19 and 21, are very much alike in appearance and size. An examination of the figures in tables 5 and 6 as well as the average values in table 7 will bear out the same fact. All of these cultures contain one part of calcium nitrate while they vary in their content of magnesium sulfate from 1 to 6 parts. It can not be said that plants of culture 1 are six times as large or six times as small as those of culture 21. This would no doubt be the case if magnesium sulfate were the salt most influential in their growth. Furthermore, culture 21 contains six times the amount of potassium phosphate as culture 1, but there is not six times as much difference in growth between the two. With an increase of calcium nitrate, however, there is an increase in vigor, weight and height of the plants. The rise in the graphs along the culture rows as brought out in connection with figures 2 and 3 also emphasizes this.

Dry weight. The figures in the second double column of table 7 giving the dry weights of plants show variations between cultures similar to those of the green weights. In general, there is little difference between the relative numbers of green and dry weight for plants of corresponding cultures.

Tuber production

The economic importance of the potato is due to its tuber and the ultimate aim of culture work is the working out of a properly balanced fertilizer that will bring about the greatest yield of tubers. With the nutrient elements contained in the three salts here used considerable differences in amounts of new tubers produced were obtained in the various cultures. The average yield of the two series is given in grams and in numbers relative to the average yield in the third double column of table 7. The greatest yield (83.5 gm.) is shown for culture 15 while cultures 10 and 18 give almost as good yields. The minimum yield of 32.7 gm. is found in culture 21. The maximum is about 2.6 times as great as the minimum. This is practically the same ratio that exists between the maximum and minimum green weights of the plants on which these tubers grew.

The relative numbers make comparisons between cultures and between these three kinds of measurements (green and dry weights of plants and green

weight of tubers) easy. There is a striking similarity between all these relative values. The average green weight of the plants is about 17 per cent heavier than the average green weight of the tubers. This relation holds good for individual cultures where the relative values are alike or nearly alike, which is the case in the majority of cultures.

The green weight of tubers of the 21 cultures is shown in figure 5 which is constructed similarly to figure 4. The seven cultures of lowest values lie
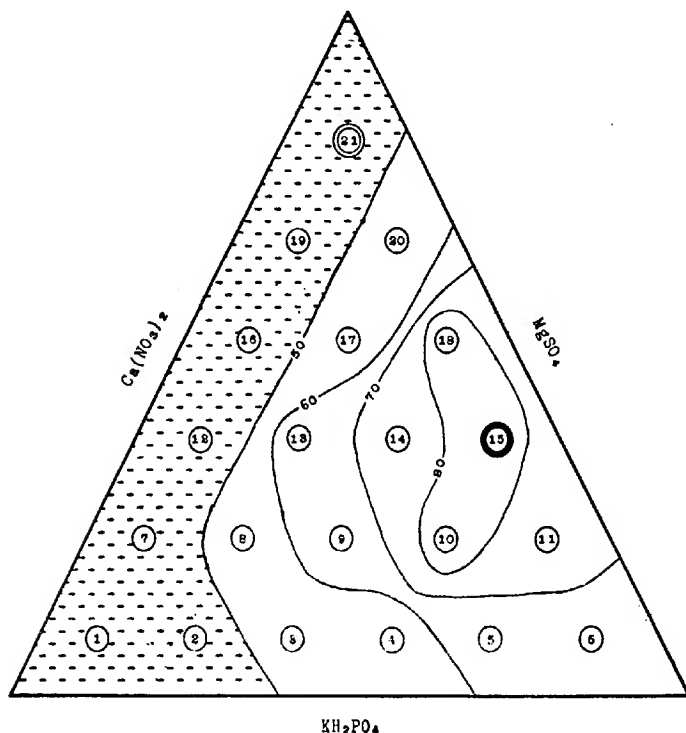
FIG. 5. DIAGRAM SHOWING THE APPROXIMATE YIELDS OF TUBERS PER CULTURE

The seven lowest-yielding cultures are within the shaded area below the 50-gm. contour line; the culture giving the highest yield is marked by the heavy circle, the lowest by the double circle.

in the "swamp land" while the contour lines are drawn for every 10 gm. of "elevation" above 50 gm. The highest culture (number 15) is encircled by a heavy ring while the lowest (number 21) is within the double ring. These two cultures are the same high and low ones of figure 4. There are minor differences in the shape of the contour lines, but in general there is marked

similarity between these two figures. Apparently the same proportions of salts that bring about good growth of plants (tops and roots) bring about good growth of tubers.

Height

The average height of plants in series I is 4.0 cm. while that in series II is 11.3 cm., or almost triple that of series I. This difference is probably due to seasonal differences of the two series.

Water requirement

The average water requirements of the cultures in the two series as given in table 7 show a maximum value of 453 for culture 20 and a minimum value of 323 for culture 15. The maximum value is approximately 1.4 that of the minimum, or there is a variation of about 40 per cent between maximum and minimum. This is a small variation when those of the other data in the table are considered. It also happens that culture 15 has the lowest water requirement and the highest green weight values of plants and tubers, but when the average water requirement values are arranged in descending order and plotted with the green weights of plants and tubers, culture for culture, there is apparently no relation seen. There seems to be a tendency for the water requirement value to remain constant under the various climatic conditions and in the various cultures of these experiments.

SUMMARY

The results obtained from two series of experiments dealing with the nutrient requirements of the Irish Cobbler potato plant are presented in this paper. Potato sprouts separated from their seed pieces were grown in sand cultures and treated with solutions designated as type I. This series of solutions consisted of 21 different salt proportions of monobasic potassium phosphate (KH_2PO_4), calcium nitrate $(Ca(NO_3)_2)$ and magnesium sulfate $(MgSO_4)$. The partial osmotic pressure of each varied by equal increments of one-eighth of the total osmotic pressure which was approximately 1.00 atmosphere.

Whether the green weight of plants (tops and roots together) or the green weight of new tubers produced is used as the criterion of growth, the best average values of corresponding cultures of the two series occurred for the cultures high in calcium nitrate and low in magnesium sulfate with a medium amount of potassium phosphate. Cultures giving the lowest yields were low in calcium nitrate. The average highest yielding culture was IR_3S_4 with the three salts in the following volume-molecular concentration: KH_2PO_4, 0.0065 M.; $Ca(NO_3)_2$, 0.0086 M.; $MgSO_4$, 0.0021 M. The average lowest yielding culture was IR_6S_1 with the following volume-molecular concentration: KH_2PO_4, 0.0145 M.; $Ca(NO_3)_2$, 0.0024 M.; $MgSO_4$, 0.0024 M.

The average water requirement for plants of the two series was 403. There was a marked tendency for individual cultures not to vary greatly from this value. There was apparently no relation between high yield and low water requirement and low yield and high water requirement.

REFERENCES

(1) BRIGGS, LYMAN J., AND SHANTZ, H. L. 1911 A wax seal method for determining the lower limit of available soil moisture. *In* Bot. Gaz., v. 51, no. 3, p. 210–219.

(2) FREE, E. E. 1915 A relative score method for recording comparisons of plant condition and other unmeasured characters. *In* Plant World, v. 18, no. 9, p. 249–256.

(3) HIBBARD, R. P. 1917 Physiological balance in the soil solution. Mich. Agr. Exp. Sta. Tech. Bul. 40.

(4) HOAGLAND, D. R. 1919 Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. *In* Jour. Agr. Res., v. 18, no. 2, p. 73–117. (Literature cited, p. 114–117.)

(5) JOHNSTON, EARL S. 1919 Nutrient requirement of the potato plant. *In* Plant World, v. 22, no. 11, p. 329–336.

(6) LIVINGSTON, BURTON EDWARD 1915 Atmometry and the porous cup atmometer. *In* Plant World, v. 18, no. 2, p. 21–30; no. 3, p. 51–74; no. 4, p. 95–111; no. 5, p. 143–149.

(7) MCCALL, ARTHUR G. 1916 Physiological balance of nutrient solutions for plants in sand cultures. *In* Soil Sci., v. 2, no. 3, p. 207–253. (Literature cited, p. 251–253.)

(8) MCCALL, A. G., AND RICHARDS, P. E. 1918 Mineral food requirements of the wheat plant at different stages of its development. *In* Jour. Amer. Soc. Agron., v. 10, no. 3, 128–134.

(9) SCHREINER, OSWALD, AND SKINNER, J. J. 1910 Ratio of phosphate, nitrate and potassium on absorption and growth. *In* Bot. Gaz., v. 50, p. 1–30.

(10) SCHREINER, OSWALD, AND SKINNER, J. J. 1918 The triangle system for fertilizer experiments. *In* Jour. Amer. Soc. Agron., v. 10, no. 6, p. 225–246.

(11) SHIVE, JOHN W. 1915 A study of physiological balance in nutrient media. *In* Physiol. Res., v. 1, no. 7, p. 327–397. (Literature cited, p. 396–397.)

(12) SHIVE, JOHN W., AND MARTIN, WILLIAM H. 1918 A comparison of salt requirements for young and for mature buckwheat plants in water cultures and sand cultures. *In* Amer. Jour. Bot., v. 5, no. 4, p. 186–191.

(13) SKINNER, J. J. 1908 Water culture method for experimenting with potatoes. *In* Plant World, v. 11, no. 11, p. 249–254.

(14) TOTTINGHAM, WILLIAM E. 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *In* Physiol. Res., v. 1, no. 4, p. 133–245. (Literature cited, p. 242–245.)

PLATE 1

CULTURES OF SERIES II ARRANGED IN THE FORM OF A TRIANGLE

Cultures on the left side are low in calcium nitrate, those on the right side low in magnesium sulfate and those on the base of the triangle low in monobasic potassium phosphate.
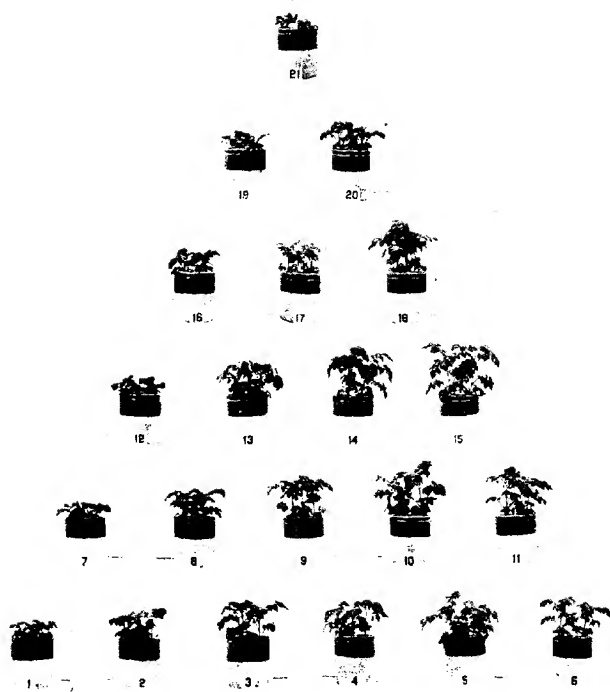
NITROGEN FIXATION BY COWPEAS AND NODULE BACTERIA

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INTRODUCTION

In studying the subject of nitrogen fixation by various legumes and nodule bacteria, it became evident that special studies of the initial appearance of the process measured chemically, and chemical studies of the mechanism of the reaction concerned, were desirable before progress could be made in other studies of an allied nature. The relation of the initial appearance of the fixation to the plant development was desired, as well as the amount fixed at early stages. The progress of the fixation was studied in order to determine whether or not it followed any special laws.

The chemistry of the mechanism of nitrogen fixation by legumes and nodule bacteria is unknown. An attempt was made to seek further data on that point.

INITIAL APPEARANCE AND PROGRESS OF NITROGEN FIXATION BY COWPEA SEEDLINGS AND NODULE BACTERIA

It was earlier observed (1) that a measurable amount of nitrogen was fixed by cowpeas in some cases as early as 14 days after planting. The present work was planned to study the fixation, if possible, at earlier periods. Consequently, experiments were initiated with cowpeas growing under especially controlled conditions. These experiments were in progress at various intervals during 4 years.

Experimental methods

The amount of nitrogen fixed by inoculated seedlings, to be significant, should be checked against the nitrogen content of seeds of similar weight, or against the nitrogen content of uninoculated seedlings subjected to the same growing conditions. Neither method can be relied upon entirely, although in most cases it would appear that the uninoculated plants would represent the most reliable check. This is not true when the uninoculated plants, because of a lack of nitrogen, decrease in their growth, and often at certain ages contain less nitrogen than the seeds of the same weight.

The average nitrogen content, as determined by the individual analyses of a reliable number of seeds of the same weight as those planted, was always

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obtained in connection with the experiment, and as seen later, was used occasionally for calculating the fixation. This established the natural variation in nitrogen content for seeds of the weight planted. The average nitrogen content of the uninoculated seedlings grown from the seeds under the same conditions of culture served as the check if it was greater than that of the seeds. The experimental error was reduced to such a small figure that a slight drop in the nitrogen content of the uninoculated seedlings was easily detectable.

Preliminary experiments

In order to obtain the lowest possible variation in nitrogen content seeds of uniform weight were selected. The natural variation of uniform-weight seeds, coupled with the slight variations accompanying the analytical methods, is sufficiently large to require careful study and greatly vitiate the results of the fixation at the early periods. The advantages for this investigation of uniform-weight seeds over random-weight seeds is clearly shown in table 1.

TABLE 1

Variation in nitrogen content of uniform- and random-weight cowpea seeds

NUMBER OF SEEDS	WEIGHT VARIATION	AVERAGE WEIGHT PER SEED	AVERAGE NITROGEN CONTENT PER SEED	NITROGEN VARIATION
	mgm.	*mgm.*	*mgm.*	*mgm.*
20	185–244	201.38	6.94	3.90
18	180–228	206.00	7.11	4.86
25	185–186	185.50	6.36	2.26
40	198–202	200.00	7.11	2.19

It is also important to have the seed well matured and dry as a greater variation was found with new seeds than with samples from the same lot a few months later; however, old seeds are not desirable as the germination is usually poorer. The suggestion is made in connection with the selection of the seeds that selections from a pure line of seeds of equal specific gravity and uniform weight would possibly reduce to a minimum the natural variations occurring in the nitrogen content.

The importance of carefully purifying the sand is well demonstrated by the data given in table 2. This sand showed no nitrogen by a total nitrogen analysis. A test for nitrate showed a trace of nitrate present in the sand grains. The ability of rapidly growing nitrogen-starving plants, to obtain nitrogen where a chemical determination failed to show the amount, is brought out here. Similar results in pot-culture experiments have been found with calcium, magnesium and phosphorus.

The sand used was a high-grade, clean, washed product. The average analysis of 107 seeds of 198 to 202 mgm. weight gave 7.04 mgm. of nitrogen per seed. Tests of the seedlings grown in this sand for nitrate revealed its presence in them. The sand and seedlings failed to give the nitrate test after 13 days' growth of the seedlings.

This experiment was conducted from September 19, to October 2, 1913, and only 3 hours and 15 minutes of sunshine occurred from the time the plants broke ground until they were washed out. Because of the retarded development of the plants the increase shown by these figures over the seed analysis represents nitrate assimilated from the sand.

TABLE 2

Nitrogen fixed by cowpea seedlings in unwashed sand

AGE OF SEEDLING	NUMBER OF ANALYSES		AVERAGE NITROGEN CONTENT		AVERAGE NITRO-GEN FIXED PER SEEDLING
	Inoculated	Uninoculated	Inoculated	Uninoculated	
days			mgm.	mgm.	mgm.
7	14	14	7.58	7.61	−0.03
9	14	14	7.66	7.53	0.13
11	15	12	8.41	8.45	−0.04
13	19	17	9.08	9.07	0.01

Preparation of sand

Various methods of purification of the sand were tried. Ignition removes the nitrogen but does not leave as satisfactory a product as is desirable. The same objection was found when the sand was subjected to a reduction method with aluminum powder, or by the addition of sugar for bacterial reduction of the nitrate. The method adopted consisted of washing the sand with hot nitrogen-free distilled water until no trace of nitrate or acid could be found. One gram of precipitated nitrogen-free calcium carbonate was added per kilogram of sand. One kilogram of the prepared sand was placed in 600-cc. beakers which were covered over the top with cotton and sterilized for 4 to 6 hours at 15 pounds' pressure. After sterilization the sand was made up to 12 per cent moisture with nitrogen-free distilled water. Then 5 cc. of sterile plant-food solution prepared from nitrogen-free chemicals was added per kilogram.

Seeds planted

Cowpea seeds possessing unbroken seed-coats were selected from a large sample and weighed individually on the analytical balance. Only seeds falling within the narrow variations adopted were selected. The weights were most commonly 198 to 202 or 185 to 186 mgm. per seed. It will readily be understood that a bushel of seed would not contain a large number of seeds of these weights. A number of seeds were always analyzed from the same lot selected for planting in any given experiment. The seeds thus selected were sterilized with either alcohol or 5 per cent calcium hypochlorite solution for 2 hours, or with mercuric chloride solution, 1 to 500, for 3 minutes.

Inoculation was provided from fresh young nodules washed and crushed to avoid undue contamination. Sterile conditions were not maintained although

reasonable precautions were always taken to keep the treatments separated from each other, and from other sources of bacteria that would influence the results. The effectiveness of the method is shown by the fact that chance inoculation occurred in only three cases in all the experiments. One seed was planted in each kilogram of sand.

Samples

The seedlings were removed at various intervals. Extreme care was exercised to obtain representative samples of each lot. There is considerable danger of obtaining individuals that are more advanced in their growth than some of the others of the same age. With only one day between samplings this danger increased, especially if the weather conditions were not suitable to the plant being grown. The samples were washed out with nitrogen-free distilled water. The seed-coats, cotyledons, and seedlings were placed in a Kjeldahl flask and analyzed for total nitrogen by the Kjeldahl method, potassium bisulfate being used. Each plant was analyzed separately.

Experiment 1

The seedlings in this experiment grew very slowly and possessed an abnormal color. The roots did not develop normally. The experiment was started December 17, 1913 and terminated January 8, 1914. During this time there were only 9 hours and 35 minutes of sunshine.

TABLE 3

Nitrogen fixation by cowpeas—Experiment 1

AGE OF SEEDLING	NUMBER OF ANALYSES		AVERAGE NITROGEN CONTENT		AVERAGE NITROGEN FIXED PER SEEDLING
	Inoculated	Uninoculated	Inoculated	Uninoculated	
days			*mgm.*	*mgm.*	*mgm.*
7	18	18	7.43	7.34	0.09
9	15	15	7.32	7.59	−0.27
14	10	9	7.36	6.54	0.82
21	10	10	6.86	7.04	−0.18
21	7	4	8.25*	8.24*	

* Nitrate added.

The seeds weighed 198 to 202 mgm. and the nitrogen content was 7.32 mgm. per seed as determined by the average of 45 seeds. The results of this experiment are given in table 3. They include the age of seedlings, number of analyses of inoculated and uninoculated plants, then nitrogen content and the average amounts of nitrogen fixed per seedling.

The fixation shown for 14 days is unreliable because of the low value of the uninoculated seedlings compared with the seed. The lack of sunlight prevented a leaf growth, although the plants were old enough to show a large fixation.

Experiment 2

This experiment was started October 26, 1912. The seeds used weighed 185.6 mgm. each. In table 4 are shown the age of the seedlings, number of analyses, average nitrogen content and nitrogen fixed at each harvest. Twenty-five seeds of the same lot and weight as those planted averaged 6.364 mgm. of nitrogen each.

TABLE 4

Nitrogen fixation by cowpeas—Experiment 2

AGE OF SEEDLING	TREATMENT	NUMBER OF ANALYSES	AVERAGE NITROGEN CONTENT	AVERAGE NITROGEN FIXED PER SEEDLING
days			*mgm.*	*mgm.*
11	Uninoculated	5	6.05	−0.31
11	Inoculated	9	6.67	0.31
14	Inoculated	9	6.90	0.54
21	Inoculated	2	8.17	2.21

The results showed a fixation of nitrogen when checked against the analyses of the seeds at 14 days, which after a careful study of the variations was found reliable. Twenty per cent of all the individual analyses in this class, at 14 days' harvest, were above the average for the seeds.

Experiment 3

The advantage of experimenting during the natural growing period of the plants is well illustrated by this experiment. Warm sunny weather prevailed throughout the period from June 18 to July 9, 1914. The seeds were of 198 to 202 mgm. weight and an average nitrogen content of 7.18 mgm. was found for the 40 seeds analyzed. Germination was excellent; all plants were above ground in 4 days. Nodules were plainly visible after 7 days. In table 5 are the results obtained in this experiment.

TABLE 5

Nitrogen fixation by cowpeas—Experiment 3

AGE OF SEEDLING	NUMBER OF ANALYSES		AVERAGE NITROGEN CONTENT		AVERAGE NITROGEN FIXED PER SEEDLING
	Inoculated	Uninoculated	Inoculated	Uninoculated	
days			*mgm.*	*mgm.*	*mgm.*
6	15	15	6.71	6.98	−0.27
9	15	15	7.36	7.15	0.21
12	15	15	7.08	6.89	0.18
15	13	12	7.20	7.38	−0.18
19	12	12	9.44	7.01	2.43
21	10	13	12.51	6.69	5.82

The figures in the "inoculated" column and in the "fixation" column indicate very plainly at what age a decided increase in nitrogen occurred. The uninoculated were used as the check to calculate the fixation. Fluctuations at the early periods, such as those found above, occur and are explained on the basis of unequal growth rates, some seedlings fixing quite large amounts of nitrogen, even at early periods, while the uninoculated often lose nitrogen to the sand. It is easy to obtain regular increases in fixation if the intervals are sufficiently wide after 14 days. That fixation occurs much sooner is repeatedly shown by an examination of the individual analyses. It was in part this striking fact that led to this investigation. This experiment demonstrated that an appreciable fixation occurred between 15 and 19 days after planting.

Experiment 4

This experiment was started to study the daily increase from an early period until large amounts were obtained. Several plantings were made at different periods to avoid poor weather conditions, but unfortunately, aside from the first planting, the weather encountered was the worst for summer growth that occurred. Plantings were made June 23, July 17, and July 27. The uninoculated seedlings averaged 7.11 mgm. of nitrogen, which figure was used to calculate the fixation for the June 23–July 20 planting. The harvest at 13 days must have included especially advanced individuals, although this very danger in sampling was understood and every precaution taken to avoid it. A very reliable fixation occurred at 13 days and at each period thereafter. The fixation suddenly increased between 18 and 19 days, and practically doubled each day following. A sudden increase also was noted as occurring on or before the 19 days in the previous experiment. The results of this experiment are arranged in table 6.

The fixation found from the first planting was positive in every case. The fluctuations are no doubt, due to the fact that all the plants were so good, that some advanced ones were obtained on the thirteenth day. These figures represent a fixation beyond error. Attention is called to the fact that at 26 days after planting the nitrogen contained in the plants is about four times that originally contained in the seed, or about three times as much nitrogen has been fixed as the seed contained. It is apparent that in all three experiments a very large increase in the rate of fixation occurred at 18 to 19 days.

The results of experiments 6 and 7 are averaged in table 7. The average figures, 7.12 mgm. for the uninoculated plants, represents 174 determinations. The average for 235 seeds of the 198 to 202 mgm. weight is 7.107, or very nearly the same as for the uninoculated, proving the exactness of the experimental methods employed.

The increase in fixation does not appear to follow a mathematical progression, although it roughly approached a geometrical progression at certain times.

TABLE 6

Progressive increase in nitrogen fixation by cowpeas

AGE OF SEEDLING	TREATMENT	NUMBER OF ANALYSES	AVERAGE NITROGEN CONTENT	AVERAGE NITROGEN FIXED PER SEEDLING
		(June 23–July 20)		
days			*mgm.*	*mgm.*
13	Inoculated	10	7.79*	0.68
14	Inoculated	14	7.30	0.19
15	Inoculated	14	7.49	0.38
16	Inoculated	10	7.39	0.28
17	Inoculated	10	7.79	0.68
18	Inoculated	10	7.76	0.65
19	Inoculated	10	8.62	1.51
20	Inoculated	10	9.77	2.67
21	Inoculated	10	11.59	4.48
23	Inoculated	9	16.95	9.84
26	Inoculated	8	28.03	20.92
		(July 17–August 3)		
14	Inoculated	10	7.00†	0.15
15	Inoculated	10	7.85	1.00
16	Inoculated	8	7.48	0.63
		(July 27–August 13)		
15	Inoculated	10	6.75†	−0.10
16	Inoculated	10	7.29	0.44
17	Inoculated	10	7.56	0.71

* Uninoculated averaged 7.11 mgm.

† Seeds averaged 6.85 mgm.

TABLE 7

Increase in nitrogen fixation by cowpeas, average of experiments 5 and 6 (June 11–August 13)

AGE OF SEEDLING	NUMBER OF ANALYSES	AVERAGE NITROGEN CONTENT		AVERAGE NITROGEN FIXED PER SEEDLING
		Uninoculated	Inoculated	
days		*mgm.*	*mgm.*	*mgm.*
6	15	7.12	6.71	−0.41
9	15	7.12	7.36	0.24
12	15	7.12	7.08	−0.04
13	10	7.12	7.79	0.67
14	24	7.12	7.17	0.05
15	47	7.12	7.33	0.21
16	33	7.12	7.38	0.26
17	20	7.12	7.68	0.56
18	10	7.12	7.76	0.64
19	22	7.12	9.07	1.95
20	10	7.12	9.77	2.65
21	22	7.12	11.49	4.37
23	9	7.12	16.85	9.73
26	8	7.12	28.03	20.91

DISCUSSION ON NITROGEN FIXATION BY COWPEA SEEDLINGS

The early appearance of nitrogen fixation in cowpeas is influenced by the rate of development of the seedlings. From the observations made it appeared that temperature was an important factor in hastening the fixation. The fixation proceeded at an increasing rate, which appeared to be closely controlled by the available supply of carbohydrates. When the plants possessed only two leaves (cotyledon leaves) they were able to furnish only a very limited amount of energy-building material. The development of a third leaf is indicative of an increased supply of carbohydrate and was followed in these experiments by a rapid increase in the amount of nitrogen fixed. This is shown by table 7, in which it is seen that in three experiments there was a sudden increase in the nitrogen fixed at 19 days, while the records show that the third leaf appeared at 14 days and had become well developed on the 16th day. It should be pointed out that the third leaf (first real leaf) is trifoliate, being composed of 3 leaflets, while the first two leaves are single. This third leaf makes a very material addition to the carbohydrate laboratory of the young plant.

The utilization of the available energy of the sun's rays is more advantageous during the long days than during the short ones. More products are synthesized during the long days and consequently more are translocated and rendered available to the bacteria in the nodules. This results in a greater amount of nitrogen being fixed by the bacteria and a larger nitrogen requirement by the plant to balance its needs.

A number of other experiments were conducted but are not reported because the bad weather conditions prevailing prevented the growth of the plants. In one experiment the cowpea seeds were split into two parts, one-half of the seed removed, and the other half planted. This was tried to test the effect of robbing the seedling of a large part of its nitrogen, on the time of appearance of nitrogen fixation. The plants grew normally and the analysis indicated an earlier fixation, but the extent of data was not sufficient to warrant a detailed report.

In examining the individual analyses of the seeds, the inoculated, and the uninoculated seedlings, it was evident that for fixing nitrogen certain seedlings were superior to others of the same lot. Even at early periods occasional seedlings were found that fixed from 0.5 to 1 mgm. above the average for the particular harvest.

PRELIMINARY CHEMICAL STUDIES OF THE MECHANISM OF NITROGEN FIXATION

Plants were grown in pure nitrogen-free sand for the purpose of testing the plant juice, the nodules, leaves, stems, and roots for ammonia, nitrite and nitrate. Extreme care was exercised to have the conditions of growth free from outside sources of nitrogen. In preparing for these studies analysis of Jena beakers, earthen jars, porcelain pallets, sand, plant-food solutions, seeds, and the water, were made for nitrite and nitrates, and some for ammonia.

Plants were grown in preliminary experiments with and without nitrate in order to test the sensitiveness of the culture method and the reliability of the reagents to be employed. Interfering substances were guarded against. No flames were used in the room a day or so before testing.

The opportunity to test many hundreds of cowpea plants was presented in connection with the experiments on nitrogen fixation. A drop of the plant juice from the stem and roots of these plants was tested in a porcelain pallet for nitrite and nitrate with diphenylamine and brucine, for nitrite with α-naphthylamine suphanilic acid, and for ammonia with Nesseler's reagent. As soot particles were found heavily laden with nitric acid at most times in the vicinity of the chemical laboratories, soot and other impurities from the air were carefully prevented from gaining entrance during the testing..

Soybean and cowpea plants were grown especially to obtain large nodule, root, stem, and leaf samples at various ages. It became a regular laboratory procedure to test plants, and parts of plants, for those forms of nitrogen. Plants of many kinds grown in the greenhouse and in the field were likewise tested for comparison. In no case where inoculated legumes or parts of the same were grown under controlled conditions in nitrogen-free sand, and every other precaution exercised to eliminate these forms of nitrogen as impurities, was a positive test found for ammonia, nitrite or nitrate. In every case, where nitrate was introduced the characteristic reaction was given. It was found in all parts of the plants, at certain times, depending upon the amount added and the maturity of the plants. Plants growing in soil that were about mature failed to show nitrate. Whenever a reaction for ammonia or nitrate was obtained the source of the impurity was successfully determined. The fact that nitrate and nitrite were absent in the plants tested, permits the conclusion that it is not a product of the reaction of nitrogen fixation. It may be that the sensitiveness of the reagents used was not sufficient to detect small enough quantities. The ease with which very small quantities of nitrate were detected when it had been added, would seem to eliminate this possibility. A large sample of nodules, taken from cowpeas at a time of rapid fixation, failed to show nitrate or ammonia. This strengthens the view that inorganic nitrogen of these forms is not concerned in the symbiotic fixation of atmospheric nitrogen by legumes and nodule bacteria.

The results reported here are in agreement with our earlier results and only add more data of a negative character as to the nature of the reaction. It appears from the data obtained that the reaction is organic in nature rather than inorganic.

SUMMARY

1. The first appearance of nitrogen fixation as detected in inoculated cowpeas growing in purified nitrogen-free sand was 9 days after planting. At 11 and 12 days, a positive fixation was found but it increased more at 13 and 14 days.

2. The progress of nitrogen fixation is related to the development of the plant. The more rapidly the plant grows the greater is the rate of increase in nitrogen fixed. A rapid increase occurs soon after the time the third leaf (first real leaf), which is made up of three leaflets, is developed. This was found to be 19 days after planting in three different experiments conducted during the natural growing period of the plant.

3. By 26 days after planting the nitrogen fixed was three times that contained in the seeds planted.

4. The experiments represent 1056 nitrogen determinations, 510 of which were made on the inoculated seedlings, 294 on uninoculated seedlings, and 252 on the seeds.

5. Preliminary studies of the mechanism of nitrogen fixation did not indicate that oxidation or reduction was concerned in the process.

REFERENCE

(1) WHITING, A. L. 1914 A biochemical study of nitrogen in certain legumes. Ill. Agr. Exp. Sta. Bul. 179.

A STUDY OF THE BEHAVIOR OF CARBON DISULFIDE WHEN INJECTED INTO THE SOIL AND ITS VALUE AS A CONTROL FOR THE ROOT-FORM OF THE WOOLLY APPLE APHIS

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INTRODUCTION

The American economic entomological literature is replete with fragmentary notes and recommendations regarding the use of carbon disulfide as a control measure for the woolly apple aphis. These notes are of a conflicting nature, and can hardly serve as the basis of either a positive or a negative recommendation regarding the use of this material. In view of this, it was deemed advisable to carry out a series of carefully planned experiments with carbon disulfide, under as great a range of conditions as possible, and thereby determine the positive or negative value of this treatment. Although from the standpoint of woolly-aphis control the results were largely negative, they are believed to be of sufficient interest, considering the lack of adequate knowledge on the subject, to warrant their publication. The data presented on the behavior of carbon disulfide in the soil may also prove of value to those investigating the control of other soil-inhabiting insects or other phases of soil fumigation.

The experimental work on the control of the woolly apple aphis, as reported in this paper, was carried out in Maryland, Virginia and West Virginia during the seasons of 1915 and 1916. Root infestation by the woolly apple aphis is severe throughout this region. In addition, there are several distinct soil types. These conditions render this section of the country ideal for experimental work in the control of the root form of the woolly apple aphis.

PLAN OF THE WORK WITH THE INJECTION METHOD OF USING CARBON DISULFIDE[1]

There were three points to be determined with regard to the injection method of using carbon disulfide as applied to the control of the woolly apple aphis:

1. The best time during the year for employing the treatment.

2. Factors influencing the diffusion of carbon disulfide in the soil.

3. The question of injury to the tree resulting from the use of carbon disulfide.

[1] For a report of experiments with this material used in water, see Leach (3).

Before entering into a discussion of the factors influencing the action of carbon disulfide gas in the soil, it will be necessary to describe the methods and apparatus employed in obtaining these data and also the conditions under which these methods were evolved.

Except when employed for injury tests, it was found impracticable to use apple trees, for the following reasons:

1. Nothing was definitely known regarding the possibilities of injury to apple trees when treated with carbon disulfide. In carrying out the work, therefore, using the trees in privately owned orchards would have involved too great a risk of irreparable damage.

2. The infestation of the roots of the average apple tree by the woolly aphis is not uniform. It may be entirely localized about the base of the tree, or be all on one side of the tree, etc.

3. To determine the degree and location of the infestation of the roots of the individual tree requires a careful examination of the root system by digging, with consequent disturbing of the natural soil conditions. A tree with its root system disturbed in this fashion cannot be used for experimental work if dependable data are to be secured.

4. To employ a tree for a given experiment, knowing nothing definite regarding its degree of root infestation, results in a great deal of fruitless labor, since many trees will be uninfested or only partially infested; a condition which cannot be ascertained from an examination of the tree above ground.

The tube method

In view of the above facts it was essential that an artificial but nevertheless dependable method be evolved for studying the action of carbon disulfide upon the woolly aphis in the soil. An observation made early in the course of the work with the woolly aphis led to the perfecting of a reliable method.

It was found that the root form of the woolly aphis could be maintained in a normal condition on pieces of roots detached from the tree and kept moist. If, during the hottest period of the summer, infested roots, not too badly decomposed, are selected and buried properly in the ground, the aphids on these roots will be found alive and breeding when examined 18 days later.

Taking this observation as a basis, tubes were made to hold the fragments of infested roots described above in order that they might be easily introduced into the soil.

These tubes (plate 1) were made of wire screening (12 meshes to the inch), measuring $1\frac{1}{4}$ inches in diameter and 7 inches in length; they were provided at one end with a removable stopper to permit the introduction of the infested roots, while the other end was plugged permanently with a cork stopper, held with tacks driven through the wire netting.

To carry out an experiment dealing with the diffusion of carbon disulfide in the soil by means of these tubes, it is necessary only to make holes of the proper depth in the soil under consideration with a $1\frac{3}{4}$-inch crowbar. The number and spacing of the holes will vary with the nature of the experiment. The tubes are filled with fragments of aphis-infested roots, then placed in the holes prepared for them with the bar, the top of the tube being 3 inches below the surface of the soil. The hole is then carefully filled with earth and tamped down to its original compactness.

In practice it was found most convenient to fill thirty or forty of these tubes and keep them in a moist condition until placed in the ground, by placing them in a pail and covering with a moist burlap bag.
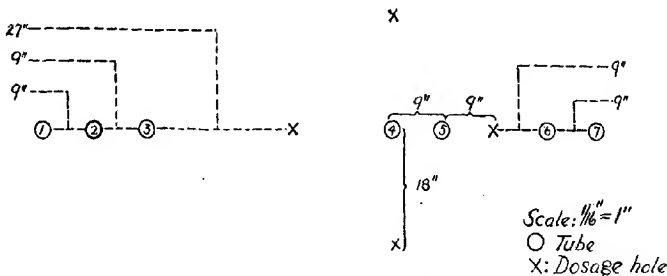
FIG. 1. THE FOUR-HOLE METHOD OF INJECTING CARBON DISULFIDE

Figure 1 shows the method of studying the diffusion of carbon disulfide gas in the soil by means of these tubes. Tube 4 would ordinarily represent the position of the base of a tree treated in practice. About it at distances of 18 inches are grouped the four dosage holes represented by X. The tubes no. 1 to 7 are so placed as to determine the diffusion in the area within the dosage holes and also the area outside and surrounding the dosage holes. The spacing of the tubes is shown in the figure.

The analogy between the tube method of studying the diffusion of carbon disulfide gas in the soil, as described above, and the treating of a tree actually infested with woolly aphis, is apparent. The dosage holes are spaced the same in both cases, while the tubes containing the pieces of infested roots serve in lieu of the actual root infestation of the tree. Furthermore, any uncertainty regarding adequate infestation is done away with, since the tubes are placed exactly where infestation is desired.

From time to time, diffusion tests were carried out on actually infested trees as a check upon the results obtained with the tube method. The tests confirmed absolutely the results obtained by the latter method and placed it above suspicion as a simple, sure method of studying the action of carbon disulfide in the soil.

The tube method as outlined above was used throughout in the experiments described in the following pages, and carried out for the purpose of determining as completely as possible, the factors influencing the diffusion of carbon disulfide in the soil. For this reason, only the conditions surrounding the individual experiment and the results and deductions obtained will be given.

Temperature records

One of the points to be determined during the course of the work was the relation of the soil temperature to the diffusion of carbon disulfide in the soil. Since it was impossible to obtain soil thermographs on account of disturbed conditions abroad, the apparatus here described was used and proved satisfactory. It consists of a wooden tube, 18 inches long, with one end open and the other covered with fine wire screening; the inside dimensions are $\frac{1}{2}$ by 1 inch, so that a maximum or minimum thermometer may be inserted and the end plugged lightly with cotton. Two of these tubes, one for the maximum and one for the minimum thermometer, were buried horizontally in a trench 9 inches deep and 30 inches long, in the soil under observation. Every 24 hours the soil about the ends of the tubes was dug away so as to allow for the removal of the thermometers from the tubes; the temperature records were taken, the thermometers adjusted and immediately replaced and the soil packed back as before. In adjusting the maximum thermometer, before placing it back in the soil tube, the bulb encased in a thin layer of cotton was dipped in carbon disulfide the rapid evaporation of which ran the mercury 20 or 30 degrees below the then prevailing soil temperature. This was found necessary because during the greater part of the time the atmospheric temperature was higher than the soil temperature.

The method of recording the soil temperature outlined above proved satisfactory from the standpoint of use in the field, the results being consistent throughout; and while there may be some error, it is not considered great enough to influence the final result. Furthermore, the seasonal soil temperatures obtained by this means compare favorably with the records obtained by others employing other types of apparatus.

Soil moisture factor

Another point to be determined during the course of the work was the effect of soil moisture upon the diffusion of carbon disulfide. With this end in view, soil samples were taken daily during the course of the experiments. In taking a sample, the dry surface soil was removed, and soil to the depth of 12 to 15 inches was obtained by means of a soil auger. These samples were preserved in air-tight jars and the moisture content later determined, the official method being used (1).

RÉSUMÉ ON THE DIFFUSION OF CARBON DISULFIDE

Gastine and Couanon (2) discuss the diffusion of carbon disulfide as follows:

Introduced into the soil in the liquid form, carbon disulfide tends immediately to saturate with its vapors the layers of soil-air which are in contact with it. Around and at the bottom of the injection hole, this saturation is produced at the end of a few moments, but it is entirely local, it can diffuse but slowly in proportion as the distance from the point where the carbon disulfide has been deposited increases. It is by gaseous diffusion that gradually the layers of soil air richest in carbon-disulfide gas cede to the adjacent soil-air layers, the toxic vapors with which they are impregnated. This exchange permits of the first becoming again saturated, from contact with the liquid carbon disulfide, with new quantities of vapor and successively the tension of the toxic product is gradually diffused in the soil-air.

While this phenomenon is being produced, there is a continual loss at the surface of the soil. The layers of soil-air adjacent to the soil surface and consequently to the atmosphere itself, constantly cede to the latter the fumes of carbon disulfide which they contain; so that, after some time, when the liquid dose confined in the soil has ceased to volatilize and when the gradual saturated diffusion can no longer be maintained, the moment finally arrives when all trace of carbon disulfide vapors disappear from the soil.

The facts are based on well-recognized physical phenomena: (a) The tension of vapors, (b) the diffusion of gases.

A volatile body constantly tends to evaporate up to that point at which the vapors accumulate and become saturated at the prevailing temperature and pressure. In the soil this state of saturation is never attained because of the continual loss to the atmosphere, a loss which results from the property possessed by the gases of penetrating and mixing intimately in spite of the considerable differences in their densities.

Observations on the vertical diffusion of carbon disulfide

The observations reported up to this point have considered only the diffusion of carbon disulfide in the horizontal sense, at the same depth as the deposit of the toxic product, that is to say at a depth of 38 or 40 cm.

In studying the presence of the vapors at higher or lower levels, the following facts are observed: the nearer one approaches the surface of the soil, the less carbon disulfide vapor is found, a fact explained by the continual loss which takes place from the upper layers of the soil in contact with the atmosphere.

In examining the soil-air below the level of the injection hole, this rapid reduction in the quantities of vapors of carbon disulfide present, is not observed. Their presence in sufficiently abundant quantity is proved in the soil-air at a depth of 1 meter or more. The diminution of the loss in proportion to the distance below the surface of the soil, the tendency possessed by the vapors of carbon disulfide, of which the density is greater than that of air, to descend, during the first moments of their emission, are the reasons for this difference.

Relative efficiency of carbon disulfide when used in the various seasons

Three sets of tests to determine the relative diffusion of given doses of carbon disulfide when employed in spring, summer and fall were carried out, two soil types being employed. During these diffusional experiments, the only factor or condition which varied to any extent was the soil temperature; the tests being so arranged that the physical conditions and the moisture content were about the same for each soil-type throughout.

The soil types

The soil type at Springfield, W. Va., employed in one-half of these experiments is known as the Berks shale loam, described by the United States Bureau of Soils as follows:

The soil of this type is grayish-brown silt loam to a depth of 6 to 8 inches. The subsoil is a pale yellow silt loam which grades into a yellowish brown or mottled yellow and gray friable silty clay. Distributed over the surface and mixed wth soil and subsoil are large quantities of small, thin, platy shale fragments, which make up from 15 to 50 per cent of the surface soil. The partly disintegrated shale is reached at a depth of 10 to 24 inches. The soil material is derived by weathering from the shale.

The soil type at Winchester, Va., employed in the second half of these experiments is known as the Hagerstown clay loam, described by the Bureau of Soils as follows:

This type has a brown to reddish-brown loam or silty loam soil and a reddish-brown to red friable clay upper subsoil which is underlain below 18 inches by a stiff red clay. It occupies undulating to rolling valley land, and drainage is good. The type is derived by weathering from pure massive limestone.

EXPERIMENTAL RESULTS

In each set of these experiments, the plat of ground employed had not been plowed or cultivated for some time previous to the beginning of the experiments.

TABLE 1

The seasonal diffusion of carbon disulfide in two soil types

SEASON AND SOIL TYPE		¼-OZ. DOSE	½-OZ. DOSE	¾-OZ. DOSE	1-OZ. DOSE	2-OZ. DOSE	SOIL-MOIS-TURE CON-TENT	SOIL-TEMPERA-TURE RANGE	
								Mini-mum	Maxi-mum
		inches	inches	inches	inches	inches	per cent	°F.	°F.
Spring diffusion	Clay	6	9	15	18	18	17.14	54 –60	58–62
	Shale	12	18	36	27	27	19.1	44½–56	48–60
Summer diffusion	Clay	6	9	15	18	18	18.76	64 –73	79–84
	Shale	15	18	36	27	27	19.43	60 –69	65–79
Fall diffusion	Clay	6	9	12	18	18	20.52	43 –48	47–53
	Shale	15	18	36	27	36	19.25	40 –48	44–50

Table 1 shows the seasonal diffusion in a clay soil and a shale soil when various doses of carbon disulfide are employed. The soil-moisture content and the range of maximum and minimum soil temperature during the course of the experiments also are given.

With one or two minor exceptions, the diffusion secured from the employment of a given dose of carbon disulfide in a specific soil type was the same throughout the three seasons of the year comprising the annual period of plant growth, irrespective of the variation in soil temperature. This was especially true with regard to the comparatively smaller doses, as, for instance, $\frac{1}{2}$ or $\frac{3}{4}$ liquid ounce per dosage hole.

Factors influencing the diffusion of carbon disulfide in the soil

Gastine and Couanon (2) discuss the influence of soil and its physical state as factors in the diffusion of carbon disulfide as follows:

The consistency of the soil and its physical condition at the time of the treatment, have a very important influence on the diffusion of the vapors of carbon disulfide.

In a general way, it may be said that in permeable soils, the diffusion is most rapid, but the permanence of the vapors is, at the same time, the feeblest. On the contrary, in compact soils, the diffusion is slow and the permanence of the vapors sometimes becomes too durable.

The most favorable soils are those in which the permeability is maintained in the subsoil, but which are capable, under climatic influences, of assuming on the surface, a certain cohesion. The diffusion of the vapors of carbon disulfide can then be effected freely without a too active loss taking place from the surface of the soil.

But whatever the nature of the soil, be it excessive permeability or too great compactiveness, it will be possible to find in either case, at certain times during the year, conditions favoring the subterranean diffusion of the vapors, and their maintenance in the soil-air.

In clay soils, care must be taken to avoid an excess of soil water which renders the soil compact and prevents the circulation of the vapors. Dryness of the soil must also be guarded against, since it gives rise to excessive permeability without any value in aiding the penetration of these same vapors.

In calcareous and light soils, a suitable hygrometrical condition is necessary to retain the vapor. A rain seals the upper soil layers sufficiently to form an obstacle to their too rapid escape, and assures the success of the operation.

The influence of soil type on diffusion

In addition to the data regarding this point given in table 1, data were obtained also with Sassafras silt loam, and Norfolk fine sand, at Berlin, Md. The former is described as follows by the Bureau of Soils:

The soil of this type to a depth of 8 to 10 inches consists of a light brown mellow silt loam containing a considerable percentage of very fine sand. The subsoil is a light brown or yellowish-brown compact silt loam. The type is derived from marine deposits weathered under good conditions of drainage.

The Bureau of Soils gives the following description of the Norfolk fine sand:

This type consists of a gray fine sandy loam, underlain by a yellow sticky fine sand or fine sandy loam. It occurs on level to undulating areas and drainage is usually good.

Table 2 shows the summer diffusion of the various doses of carbon disulfide in these four soil types, with the soil moisture content and the range of soil-temperature during the experiments.

It will be observed from table 2 that with one exception a greater diffusion was obtained on Berks shale loam with each of the five doses than was obtained on the other three soil types. In the case of the ¾-ounce dose of carbon disulfide, a diffusion of 36 inches was obtained on Berks shale loam, or twice the diffusion secured on the other soil types. The diffusion on the four soil types when a 2-ounce dose was used was fairly constant.

TABLE 2

The comparative diffusion in four soil types

SOIL TYPE	¼-OZ. DOSE	½-OZ. DOSE	¾-OZ. DOSE	1-OZ. DOSE	2-OZ. DOSE	SOIL-MOIS-TURE CON-TENT	SOIL-TEMPERA-TURE RANGE	
							Mini-mum	Maxi-mum
	inches	*inches*	*inches*	*inches*	*inches*	*per cent*	*°F.*	*°F.*
Berks shale loam....................	15	18	36	27	27	19.43	60–69	65–79
Hagerstown clay loam......,........	9	9	18	18	27	10.43	70–76	80–83
Sassafras silt loam..................	9	9	18	18	27	20.13	68–70	70–72
Norfolk fine sand..................	9	18	18	27	36	6.77	69–72	71-73

Although the above data cover but four soil types, the facts obtained in the course of the work form the basis for certain conclusions regarding the influence of soil type on diffusion:

1. It is impossible to state with certainty the degree of diffusion obtainable by the use of a given dose of carbon disulfide in a given soil type, except on the basis of direct experimentation.

2. In all probability, the thousand and one distinct soil types of different origin, grading from clay to silt, to sand and gravel, etc., would be found to have distinct differences in their penetrability by carbon disulfide.

The influence of soil moisture upon diffusion

In order to obtain data on this point, experiments were conducted on Berks shale loam and Hagerstown clay loam; when at intervals during the season they were in a wet, a moist and a dry condition. Table 3 gives the range of soil temperature and the moisture, together with the comparative diffusion.

It. will be observed that the diffusion in general was much less in wet clay than in moist or dry clay, while the results were directly opposite in the shale loam. Apparently, therefore, excessive moisture in heavy soils causes a decreased diffusion resulting from the employment of a given dose of carbon disulfide, indicating that the best time for securing maximum diffusion occurs when the soil is in a fairly moist or dry condition. An abundance of moisture,

on the other hand, is desirable in securing maximum diffusion in the light soils. These data coincide with the observations of Gastine and Couanon recorded above.

The degree of soil moisture at the time of treatment with carbon disulfide is undoubtedly the one limiting factor in securing the maximum diffusion of a given dosage of the material. It may be said of any soil type, that there is a definite range of soil moisture within which the diffusion will be greatest, and that a degree of soil moisture above or below this definite range, depending on the character of the soil type, will result in decreased diffusion with the same dosage. In this respect, soil types in general may be divided into two broad classes; the light class and the heavy class. The Berks shale loam may be taken as an example of the former and the Hagerstown clay loam as an example of the latter.

TABLE 3

Factor of soil moisture

SOIL TYPE	MOISTURE CONDITION	½-OZ. DOSE	½-OZ. DOSE	¾-OZ. DOSE	1-OZ. DOSE	2-OZ. DOSE	SOIL-MOISTURE CONTENT	SOIL-TEMPERATURE RANGE	
								Minimum	Maximum
		inches	inches	inches	inches	inches	per cent	°F.	°F.
Hagerstown clay loam.....	Wet	6	9	12	18	18	20.52	43–48	47–53
	Moist	9	9	18	18	27	10.43	70–76	80–83
	Dry	9	9	18	18	27	2.75	71–73	78–81
Berks shale loam.........	Wet	15	18	36	.27	27	19.43	60–69	65–79
	Moist	9	18	36	27	27	12.10	72–76	76–80
	Dry	9	9	18	18	27	7.55	56–64	60–69

Soil-surface moisture as an aid in securing maximum diffusion

Gastine and Couanon discuss this point as follows:

In calcareous and light soils, a suitable hygrometrical condition is necessary to retain the vapor. A rain seals the upper soil layers sufficiently to form an obstacle to their too rapid escape and assures the success of the treatment.

The experiment given in detail below, offers some interesting data on this question.

During the months of August and September, 1916, the rainfall at Springfield, W. Va., was very slight and by October first the Berks shale loam was extremely dry and hard to a very considerable depth below the surface. On the night of October third, an extremely heavy shower of short duration occurred, resulting in a total rainfall of 1.53 inches. This huge bulk of water, falling quickly upon a hard, dry soil, ran off to a great extent wherever the

ground sloped and as a result only the first two or three inches of the upper soil was moistened to any extent, leaving the soil below as dry as before the rainfall.

This set of soil conditions was admirable for determining the diffusion of carbon disulfide when the dry, hard subsoil is sealed by two or three inches of moist top-soil.

On the next morning, immediately succeeding this heavy rainfall, a series of experiments with the tube method were instituted on a plot of Berks shale loam. The ground sloped slightly, and as stated above, the top-soil was moist and the lower soil dry.

From a study of the data presented in table 4 it will be noted that the standard maximum diffusion for this soil type was secured only when the under-soil was moist, regardless of the condition of the surface soil. A soil in which the two or three inches of surface soil was moist, but the under-soil dry, resulted in minimum diffusion. The same result occurred when both the under-soil and the surface soil were dry.

TABLE 4

The influence of the several conditions of surface-soil upon the diffusion of carbon disulfide
DeKalb shale loam

SOIL CONDITION	$\frac{1}{4}$-oz. DOSE	$\frac{1}{2}$-oz. DOSE	$\frac{3}{4}$-oz. DOSE	1-oz. DOSE	2-oz. DOSE	SOIL-MOIS-TURE CON-TENT	SOIL-TEMPERA-TURE RANGE	
							Mini-mum	Maxi-mum
	inches	inches	inches	inches	inches	per cent	°F.	°F.
Surface moist, under-soil dry.........	9	9	18	18	27	8.72	50–64	65–69
Surface dry, under-soil dry...........	9	9	18	18	27	7.55	56–64	60–69
Surface moist, under-soil moist.......	15	18	36	27	27	19.43	60–69	65–79
Surface dry, under-soil moist.........	9	18	36	27	27	12.10	72–76	76–80

Apparently, the condition of the surface soil exerts less influence upon the diffusion of carbon disulfide and acts less as a check upon the dispersion of the vapors into the atmosphere, than would be ordinarily supposed. This question will be taken up in greater detail under the discussion of the "Influence of surface-soil cultivation upon the diffusion of carbon disulfide in the soil."

Influence of the spacing and arrangement of the dosage holes upon the diffusion of carbon disulfide

There are three factors in the problem of arranging the dosage holes to secure maximum efficiency when treating apple trees for the control of the root-form of the woolly apple aphis:

1. The necessity for securing an evenness of aphid mortality throughout the treated soil area.

2. The necessity of securing maximum diffusion per unit dose of carbon disulfide employed.

3. The necessity of guarding against injury to the tree resulting from the improper placing of the dosage holes.

Of these three factors, the last is the limiting one. Any arrangement of the dosage holes involving the injection of carbon disulfide in immediate proximity to the base of the tree, a procedure which invariably causes severe injury to the crown, cannot be employed, no matter how theoretically perfect it may be.

The four-hole method (fig. 1) employed almost entirely in the control of the *phylloxera* in France, is in all probability the simplest and most efficient method that can be used against the woolly apple aphis. The use of this system caused a minimum of root injury. Its efficacy is due to the fact that each individual injection of carbon disulfide is so placed as to allow for maximum diffusion and the combined result of the individual diffusions is the complete mortality of the aphids in the area of soil treated—all accomplished with the minimum quantity of liquid carbon disulfide. This may be accounted for by a discussion of the character of the diffusion from the individual injection hole.

The diffusion of carbon disulfide in the soil from a single dosage hole, not surrounded by other dosage holes, extends equally in all directions from the point of injection of the liquid. In a soil infested with woolly aphis, the area within which the aphids are killed takes the form of a circle with the dosage hole as the center.

Bearing this fact in mind, provided we know the area of diffusion which can be attained by a given dosage of carbon disulfide, it is a simple matter so to group the dosage holes about the base of a tree (fig. 1), as to allow sufficient space in order that each injection of carbon disulfide shall do the maximum amount of work.

The experimental work has definitely shown for instance, that a dose of $\frac{1}{2}$ ounce of liquid carbon disulfide in volatilizing will diffuse and produce aphid mortality for a distance of 18 inches surrounding the dosage hole in moist Berks shale loam. The circles of figure 2, drawn with the individual dosage holes as centers, show the extent of the diffusion. The areas of diffusion overlap sufficiently to insure complete aphid mortality at the extreme points where the individual diffusions meet.

This spacing of the dosage holes will remain constant but the dose will vary with the nature of the individual soil type. In case of larger trees with a greater area of infested root surface than could be properly cared for with four dosage holes, additional injection holes, properly spaced, were made.

It would appear from an examination of figure 2, that the areas *a*, *b*, *c* and *d*, overlapped by the diffusions from the individual dosage holes, would be

subjected to a too intense density of carbon disulfide fumes. It will be shown, under the discussion of the character of diffusion, that this is not the case (p. 441).
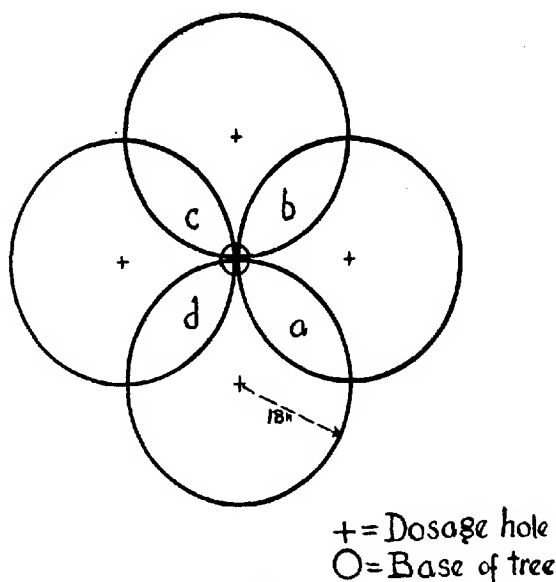
FIG. 2. AREA OF DIFFUSION FROM THE INDIVIDUAL DOSAGE HOLES

The depth of the dosage hole

The area of diffusion obtained from the employment of a given dose of carbon disulfide depends upon the depth to which the material is injected in the soil. In order to determine the most efficient depth, a series of experiments were carried out in two soil types, Berks shale loam and Hagerstown clay loam. Using the tube method previously described, the diffusion of a $\frac{3}{4}$-ounce dose of carbon disulfide was determined when deposited in dosage holes at a depth of 2, 4, 6, 8, 10, 12, 14, and 16 inches.

Figure 3 shows the comparative diffusion in moist Berks shale loam at the various depths of injection. It will be observed that the greatest diffusion occurred when the dose was placed at a depth of 6, 8, or 10 inches below the surface of the soil. At greater or lesser depth, the diffusion was much less. With a dosage hole 2 inches in depth, a diffusion of 18 inches was secured in this soil, because the liquid carbon disulfide sinks into the soil rapidly and the

soil used to plug the hole does not soak it up to any extent and thereby allow it to escape into the atmosphere. The 4-inch dosage hole gave two-thirds the diffusion of the 6, 8 and 10-inch dosage holes. When dosage holes of a greater depth than 10 inches were employed the diffusion became less with the increase in depth.
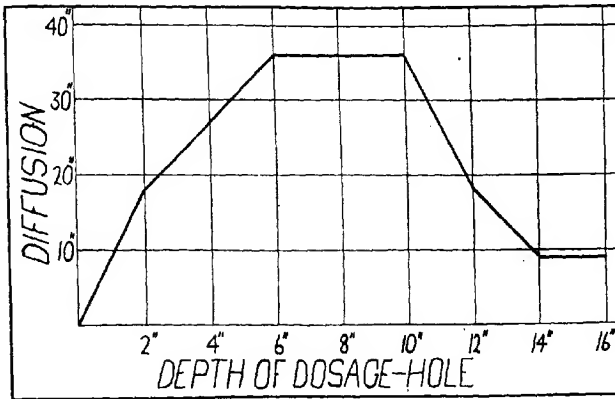
Fig. 3. Effective Diffusion of Carbon Disulfide in Berks Shale Loam When Injected at Various Depths
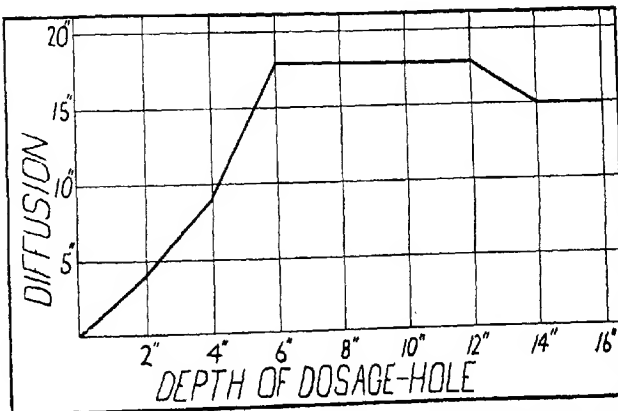
Fig. 4. Effective Diffusion of Carbon Disulfide in Hagerstown Clay Loam When Injected at Various Depths

The series of tests outlined above were duplicated on Hagerstown clay loam. Figure 4 shows the comparative diffusion in this soil type, at the various depths of injection.

The greatest diffusion occurred when the dose was placed at a depth of 6, 8, 10 or 12 inches below the surface of the soil. At greater or lesser depths the area of diffusion decreases. With a dosage hole 2 inches in depth, the diffusion obtained was negligible; because the liquid carbon disulfide does not immediately sink into the stiff clay soil, thereby suffering a loss due to evaporation. In addition; the soil used to plug the dosage hole soaks up a considerable portion of the dose, which is subsequently given off into the atmosphere. The 4-inch dosage hole proved too shallow for the maximum diffusion, while the comparatively deep dosage holes of 14 and 16 inches fell short of producing the diffusion attained at slightly shallower depths.

The fact that maximum diffusion, in both of the above soil types, is secured when the carbon disulfide is injected at a depth varying from 6 to 10 inches may be explained on the basis of the specific properties of the vapor of carbon disulfide. These vapors are more than twice as heavy as air, and as the injection of liquid carbon disulfide gradually volatilizes in the dosage hole, the fumes have a greater tendency to sink in the soil as they diffuse than to rise toward the surface. It is therefore necessary to inject the liquid at a medium depth in the soil, sufficiently deep to prevent loss through the soil cap of the dosage hole and yet sufficiently shallow to provide for an even diffusion in the upper layers of the soil. Eight inches is probably the most convenient depth from the standpoint of both maximum diffusion and the minimum of labor required in making the dosage holes.

The speed of diffusion and aphid mortality

Early in the experimental work with the injection method of using carbon disulfide it was found necessary to obtain data on the speed of the diffusion of the material in the soil, and the consequent aphid mortality resulting therefrom. This information was required because otherwise the experiments might have been examined for aphid mortality before the action of the gas in the soil was complete.

Experiments to determine this point were carried out in Berks shale loam during the hottest portion of the growing season and again in the early fall when the soil and atmosphere were cooler. The experiments were planned as follows.

Twelve individual sets of tubes containing aphid-infested roots (fig. 1), were placed in the soil, the four-hole method being used and sufficient space allowed between each set of tubes so that there could be no possible interference of diffusion. The experiment was carried out in August, and as it

happened, during the hottest period of the growing season. The plat of
ground employed had not been tilled for some time, while a soil-moisture
determination at the beginning of the experiments gave 16.10 per cent moisture,
the soil being in a very moist, plowable condition. The dosage used through-
out was ¾ ounce per injection hole. The maximum soil temperature varied
from 75 to 80°, and the minimum from 72 to 76°, during the course of the
experiment.

The cylinders containing the infested roots were all placed on August fourth
and the carbon disulfide injected August fifth. Twenty-four hours after the
injection of the carbon disulfide the first set of cylinders was examined for
aphid mortality, at the end of 48 hours the second set was examined, and each
24 hours thereafter a set of cylinders was examined until the end of the twelfth
day, when the experiment was closed.
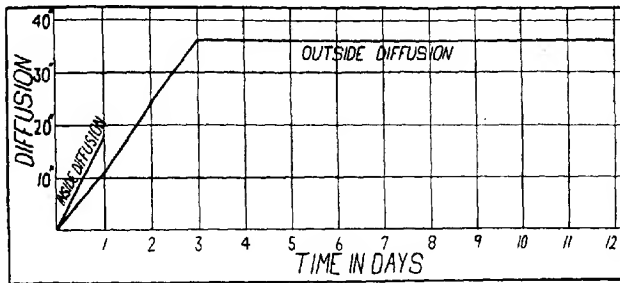
FIG. 5. SPEED OF APHID-KILLING DIFFUSION—MOIST SOIL

The data given in figure 5 represents the speed of *killing* diffusion, that is,
the time required for aphids at a given distance from the dosage hole to be
killed through exposure to the gas.

It will be observed that the inside diffusion[2] resulted in complete aphid
mortality within 24 hours, while the maximum outside diffusion and conse-
quently aphid mortality was not attained until 3 days after the injection of
the carbon disulfide.

Speed of diffusion in cool weather

By October the ground had cooled off considerably and another series of
experiments was carried out as above. Unfortunately for comparison with
the above results the soil in this instance was dry, containing only about
8.72 per cent of moisture. The maximum soil temperature varied from 63
to 69° and the minimum from 56 to 64° during the course of the experiments.
The dosage used throughout was ¾ ounce.

[2] See page 423.

The results of the experiment are given in figure 6. It will be observed that the inside diffusion was complete in 24 hours, while the outside diffusion required 3 days. The irregularity in the diffusion results obtained on the seventh day can be explained only on the basis of increased soil moisture in the special plat of ground occupied by that particular set of tubes.
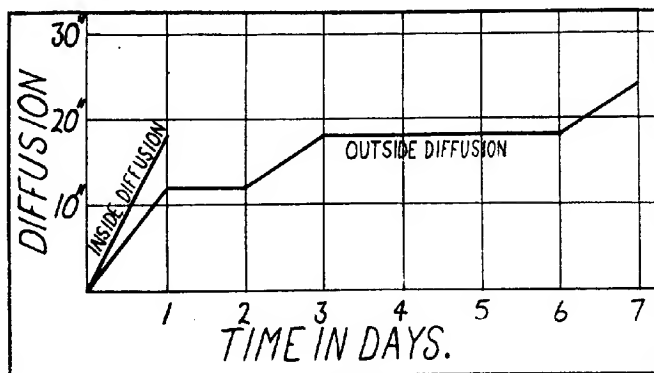
FIG. 6. SPEED OF APHID-KILLING DIFFUSION—DRY SOIL

The effect of cultivation

Gastine and Couanon (2) discuss this subject as follows:

Whatever the nature of the soil, the treatment should not be effected after cultivation, an operation which renders the soil unfit to conserve the vapors of carbon disulfide. It is the one explicit rule, practically justified both by the practical results and the scientific study of the diffusion. In cultivated soil, it is a general rule that the vapors of carbon disulfide find it easier to escape directly into the atmosphere than to diffuse in the soil-air, where nothing tends to retain them. Forty-eight hours after the treatment, one invariably finds only slight traces of carbon disulfide, even in the immediate vicinity of the dosage holes.

In spite of the above emphatic ruling, the writer was interested in obtaining further data on this point. Experiments were therefore carried out under various conditions of soil type and soil moisture, as well as other physical soil conditions.

Two sets of studies were conducted on Sassafras silt loam at Berlin, Md. The soil was in a moist condition. In the first set the ground was thoroughly dug up to a depth of 8 inches, the lumps broken and the surface smoothed with a spade. The tubes containing aphis-infested roots were placed as usual in holes made with a bar and covered with 3 inches of earth, the latter not being stamped down. The carbon disulfide, used at the rate of $\frac{3}{4}$ ounce per

injection hole with the four-hole method, was injected to a depth of 8 inches and the dosage hole thoroughly plugged with soil.   In the second set of experiments the surface of the soil was cultivated to a depth of 2 inches with a hoe, the tubes being placed and the carbon disulfide injected as above.   As a check on these two experiments, a third experiment, with the same dosage, was carried out in hard, untilled ground that had not been cultivated or disturbed for some time.

These experiments were duplicated in Berks shale loam, the soil being dry and hard, and in Hagerstown clay loam under moist conditions.

Table 5 gives the combined results of the above tests.

TABLE 5

Diffusion in untilled, tilled and cultivated ground

SOIL TYPE	SOIL CONDITION	DOSAGE	DIFFUSION
		oz.	*inches*
Sassafras silt loam..............	*Not tilled; hard and moist	$\frac{3}{4}$	18
	†Tilled; moist	$\frac{3}{4}$	18
	‡Surface cultivated; moist	$\frac{3}{4}$	18
DeKalb shale loam.............	Not tilled; hard and dry	$\frac{3}{4}$	18
	Tilled; dry	$\frac{3}{4}$	18
	Surface cultivated; dry	$\frac{3}{4}$	18
Hagerstown clay loam..........	Not tilled; hard and moist	$\frac{3}{4}$	18
	Tilled; moist	$\frac{3}{4}$	18
	Surface cultivated; moist	$\frac{3}{4}$	18

* Ground not plowed or cultivated recently.

† Soil dug to a depth of 8 inches with spade.

‡ Soil-surface cultivated 2 inches deep.

It is evident from the three distinct sets of data presented in table 5, that an undisturbed condition of the soil is not absolutely essential in securing the maximum diffusion of a given dose of carbon disulfide.

Before drawing any further conclusions from the above data, it might be well to emphasize the fact that the conditions surrounding these experiments were slightly idealized.   The tubes containing the aphid-infested roots were buried 3 inches deep in the loose cultivated soil, whereas in practice the stirring of the soil drags infested roots to the surface and loosens and partially exposes roots growing near the soil surface.   Since the density of the diffusing gas is least in these upper two or three inches of soil, it follows that an open condition of this surface soil will result in the aphids contained therein escaping the prolonged action of the fumes, thereby forming a fertile source of reinfestation.   The aphid-infested roots at lower depths and consequently sub-

jected for a greater length of time to the action of the fumes, will be cleared of the parasites, but only until the progeny of aphids on the surface roots again reinfest them.

It may be said that aphids in the two or three inches of upper soil are the most difficult to kill, even under the most favorable conditions, with the fumes of carbon disulfide, while those at lower depths are more easily killed. Disturbing the surface-soil in any way tends to produce a set of conditions unfavorable to the diffusion and retention of the vapors in these upper soil-layers, thereby allowing the escape of the aphids contained therein.

The relation of dosage to diffusion

The degree of diffusion obtained from the injection of carbon disulfide is not in direct proportion to the size of the dose employed. This is shown by the data presented in figure 7.
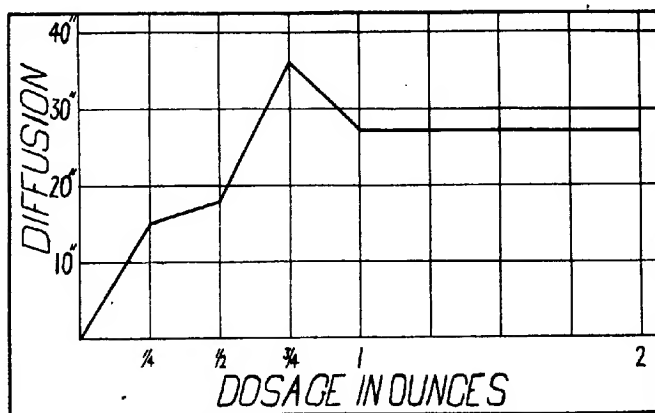
FIG. 7. THE RELATION OF DOSAGE TO DIFFUSION

It will be observed that the relatively small doses are more efficient than the larger doses. A ¾-ounce dose gave greater diffusion than 1 or 2 ounces; in fact a dose of ¾ ounce by volume is in most cases the most efficient dosage to employ.

The influence of soil temperature

The minute variations in soil and atmospheric temperature exert an influence upon the *course of the diffusion of carbon disulfide in the soil*. Marion (4) makes the following statement regarding this factor:

The curve representing the horizontal penetration of carbon disulfide figures as a sinuous line of which the apices correspond to the hours of the day, while the depressions concord with the hours of the night. It may be also stated that these oscillations reproduced exactly those of the thermometer curve, while the barometric pressure did not appear to exert any notable influence.

It is apparent that the diffusion of a dose of carbon disulfide in the soil is subject to bursts of speed and abrupt checks in speed as the temperature varies. However, within broad limits, *the temperature does not affect the ultimate area of diffusion* attained by a given dose to carbon disulfide in a given soil type, when the other influencing factors remain constant.

The data presented in table 1 show the diffusion of various doses of carbon disulfide, under practically constant conditions of soil moisture, etc., when applied at various periods during the growing season beginning at the time when the apple buds were swelling and continuing, at intervals, through the season until the growth ceased in the late fall. The effective diffusion of a given dose of carbon disulfide was the same in the heat of midsummer as in the lower temperatures of late fall. In Berks shale loam, for instance, $\frac{3}{4}$ ounce of carbon disulfide applied in August, during the hottest period of the growing season, gave a diffusion of 36 inches, and with that dosage the diffusion was the same in November. The range of soil temperature in August was: Minimum 60–69°; maximum 65–79°, and in November, minimum 40–48°; maximum 44–50°. No data on diffusion were obtained on this soil type at lower temperatures than those recorded above, but the writer is inclined to the opinion that soil treatment with carbon disulfide had best be limited to the three seasons of the year comprising the annual period of plant growth.

INJURY

The question of injury to the plant, resulting from the use of any material as an insecticide, is of the utmost importance in considering its merits. For this reason a detailed series of experiments with carbon disulfide, extending throughout the season, were carried out for the specific purpose of obtaining accurate data regarding the action of this material upon the apple trees, when injected into the soil for the purpose of controlling the woolly aphis.

It was, of course, impossible to carry out any extended series of dosage tests for injury in commerical orchards; the chances of damage were too great. Fortunately for the success of the work, the use of a block of trees was secured at the farm of the United States Insecticide Board, at Vienna, Va. The trees comprising this block were in their second year of growth at the time of the experiments; they were of the Stayman Winesap variety, and in a strong, vigorously growing condition.

The soil type was the Manor loam, described by the Bureau of Soils as follows:

The soil is a yellow or yellowish-brown loam, about 8 inches deep. The subsoil is a yellow or reddish-yellow heavy loam, which grades into clay loam; this type is derived from micaceous schists, and small mica fragments occur in soil and subsoil.

In addition, several sets of dosage tests for injury on trees of various ages were carried out during the season at Springfield, W. Va. The soil type was the Berks shale loam. As a result of the above experiment, data were obtained on the following points affecting the use of carbon disulfide, as a control for the woolly aphis:

1. The comparative degree of injury caused by carbon disulfide when applied at the different periods of the growing season.

2. The comparative amount of injury caused by various-sized doses.

3. The action of carbon disulfide upon apple trees of various ages.

4. The outgrowth of injury resulting from the use of carbon disulfide.

5. The arrangement of the dosage holes as affecting the degree of injury.

6. The degree of injury arising in various soil types.

7. The soil moisture-content as a factor in injury.

Plan of dosage tests

Beginning in April and continuing once each month throughout the season until December, trees were treated with various dosages in order to ascertain the killing dose and also the comparative effect of the various doses. The trees used for these tests were strong and vigorous, in their second year of growth. The arrangement of the dosage holes, except where otherwise stated, is shown in figure 1.

The four dosage holes were made 8 inches in depth and placed 18 inches from the crown of the tree. The doses employed per injection hole were $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1 and 2 ounces, by liquid measure, and two trees were treated with each dose at each test.

The dosage tests involved, in all, the treatment of more than 200 trees, and in view of the large number of tests it is impossible to give the date for each individual test. Instead, a general discussion of the character of the injury and the factors influencing the degree of injury is given, based upon these individual tests.

Discussion of injury

The zone of injury. The ultimate result of the action of the fumes of carbon disulfide upon the portion of the root in the vicinity of the dosage hole is to kill the cambium and thereby girdle the root, resulting in its death beyond the girdled point. Since the dosage hole must be placed within 18 inches of the

base of the tree, in order that the fumes shall penetrate and kill the aphids infesting the base, it follows that this girdling of the roots will take place mainly within a zone 6 to 15 inches from the base of the tree, depending upon the strength of the dose employed and the consequent range of injury resulting.

The unevenness of diffusion and consequent injury. Observations made during the course of the dosage tests recorded above indicate that the diffusion of carbon disulfide in the soil is uneven; in other words, the density of the gas in the soil gradually becomes less and less as it diffuses outward from the dosage hole. That is, during the course of the diffusion, the soil and roots therein at a point 9 inches from the dosage hole will be subjected to a greater density of gas for a greater length of time than will the roots at a point 18 inches from the dosage hole, etc. Direct experimentation has shown that a given dose of carbon disulfide will kill the main roots of young trees, for one-half the distance attained by the carbon disulfide gas in its path of aphid-killing diffusion. For instance a dose of $\frac{3}{4}$ ounce will result in the mortality of the aphids within a radius of about 16 to 18 inches of the dosage hole in a stiff clay soil, while any main roots within this radius will be killed for a distance of from 6 to 8 inches from the dosage hole, or on an average of one-half the radius of aphid mortality. This condition prevails whether the dose employed is $\frac{1}{4}$ ounce or 2 ounces per dosage hole. The rootlets, on the other hand, are killed for the same distance as are the aphids, and in the majority of cases, *beyond. In other words, it requires less density of gas to kill a rootlet than an aphid.* The above condition of affairs applies irrespective of the dosage employed, the injury sustained varying directly with the strength of the dose.

Injury to individual roots by the fumes of carbon disulfide, showing the gradation of injury at varying distances from the dosage hole, were observed at various times. The most typical of these is shown in plate 2, B. It will be observed that up to point *b*, the main root was killed outright. From *b* to *a*, the cambium was killed in patches, usually about the base of a rootlet. From *a* to the base of the tree only the rootlets and tips of the rootlets were killed. This typifies the gradual decrease in the density of the carbon disulfide fumes as the distance from the dosage hole increases and the consequent decrease in injury.

At this point it might be well to state that in a given soil type, under a given set of soil conditions, a certain specific dose of carbon disulfide is *necessary* in order to obtain aphid mortality in a given area surrounding a dosage hole. A smaller dose will not do the work, and while the employment of an insufficient dose will cut down the amount of injury, it will not produce the effect desired, namely, complete control.

A typical case of root injury or girdling is shown in plate 2, A. This root emanating directly from the base of the tree and 8 inches below the surface

of the soil, passes 4 inches to one side of a dosage hole. The root was about ¾ inch in diameter at the base and 28 inches long. It will be observed that the killing of the cambium extended from point *a*, the point of the root 4 inches to one side of the dosage hole, to point *b*. The distance from *a* to *b* was 9 inches. A dose of 1 ounce was applied on July 11, the tree from which the root was taken being 2 years old and growing in clay soil. The particular root shown in plate 2, A was typical of the injury resulting from the use of the above dose under the conditions prevailing at the time of treatment. The roots were killed for a distance of about 9 inches from the dosage hole while the rootlets were killed for a considerably greater distance from the hole.

Outgrowth of injury. The tree from which the root described above was taken, was injured as a result of the injection of the carbon disulfide and the top did not begin to grow again until about 5 weeks after the treatment. The roots, in the meantime, were repairing the damage caused by the action of the carbon disulfide and when examined on October 23, the root presented the appearance as shown in plate 2, A. At points *c* and *d*, the root stump had produced bunches of new, vigorous roots, designed to take the place of the dead portion—*b–a*. This is a typical instance of root growth following carbon disulfide injection. It is evident that this bunched root system so near to the base of the tree will hardly be of as great value to the tree as would the single root *b–a*, since the latter will in its growth, rapidly spread to the less crowded portions of the soil, where the struggle for food and moisture is less acute. The normal amount of growth that the single root would have enjoyed must be divided up among the nine roots emanating from the stump. Restricted in their growth, as a result of this condition, their efforts as food and water procurers will be confined to a limited soil zone, thereby resulting in a decreased tree growth.

Injury to the top. This phase of the subject will be treated under two headings, as follows:

1. Injury to the trunk and main branches.

2. Injury to the growing tips and foliage.

Injury to the trunk, branches, growing tips and foliage of the apple tree resulting from the injection of carbon disulfide might be termed secondary injury. It is indirectly due to the injury sustained by the roots. The latter, as a result of this injury, cease or partially cease to function for a time, thereby failing to supply the top with water and plant-food, and consequently checking the growth or killing portions of the top. While important, it may nevertheless be said that injury to the top is but an indication of injury to the roots, and it is there that the fundamental injury occurs.

Injury to the trunks and main branches was observed only when very excessive doses were employed in shale loam soils.

As to injury to foliage and growing tips, the first indication of injury resulting from the injection of carbon disulfide is the withering of the foliage. In the case of a large dosage, the entire foliage will wither and die within a few days after treatment. Then the tips of the branches begin to die back often for a distance of ten or twelve inches. This occurs invariably when a dosage of 2 ounces per injection hole is employed. A dose of 1 ounce per injection hole will, as a rule, strip the foliage to some extent on the tips of the branches and kill the tips back for a few inches. The terminal buds on the remaining tips will not be killed but will be checked in their growth. A dose of ½ ounce will kill one or two tips and strip the foliage very slightly. The remaining tips will be checked in their growth. A dose of ¼ ounce per injection hole will seldom cause any defoliation or killing of the tips but *will* check the growth at the tips.

The degree of injury to the aerial portion of the tree, therefore, depends upon the degree of root injury. So much of the root surface is killed when large doses are employed that the tree is not only unable to maintain the aerial growth already made, but must suffer the death of the newest and tenderest growth. In the case of medium doses, the tree suffers some defoliation and a decided check in growth, while in the case of small doses, the tree stands still and the new wood has a tendency to ripen. The effect of small doses is very similar to that of a long drouth.

Explanation of top injury. The specific cause of this phase of carbon disulfide injury is not known, but all evidence points to an interference with the normal transpiration. So much of the root system of the tree is killed that the necessary amounts of water and plant-food cannot be supplied, resulting in the killing or checking of the foliage, terminal buds and immature wood.

The relation of root-injury and interference with normal transpiration is shown in figure 8. The dosage holes *A, B, C* and *D*, were made 8 inches deep and placed 18 inches from the base of the 2-year-old tree (*O*). The dosage employed was ¾ ounce per dosage hole, injected on August 18.

As a result of the above treatment, the foliage was quite badly stripped and several of the growing tips were killed back. An examination showed the roots killed for a distance of 8 inches surrounding each dosage hole. This is shown diagrammatically in figure 8, the circles whose centers are the dosage holes, *A, B, C* and *D*, being 8 inches in radius. It will be readily seen that one-half of the roots emanating from the base of the tree, assuming that they radiate evenly, pass through the circles whose centers are *A, B, C* and *D*, in other words, through the areas in which the roots are girdled and killed. In addition, the rootlets are killed considerably beyond the area shown in the circles. This means that almost half the entire root system of the trees was rendered functionless as a result of the treatment, thereby cutting down the water supply to the leaves one-half, with the resulting injury to foliage and growing tips.

Miscellaneous factors

Age of the tree.   The age of the tree does not have such a marked influence upon the degree of injury as would be supposed.   Two parallel sets of experiments illustrating this fact were carried out at Vienna, Va., 2-year-old trees being used in one set of experiments and 5-year-old trees in the other.   The degree of injury for each respective dose was about the same for the trees
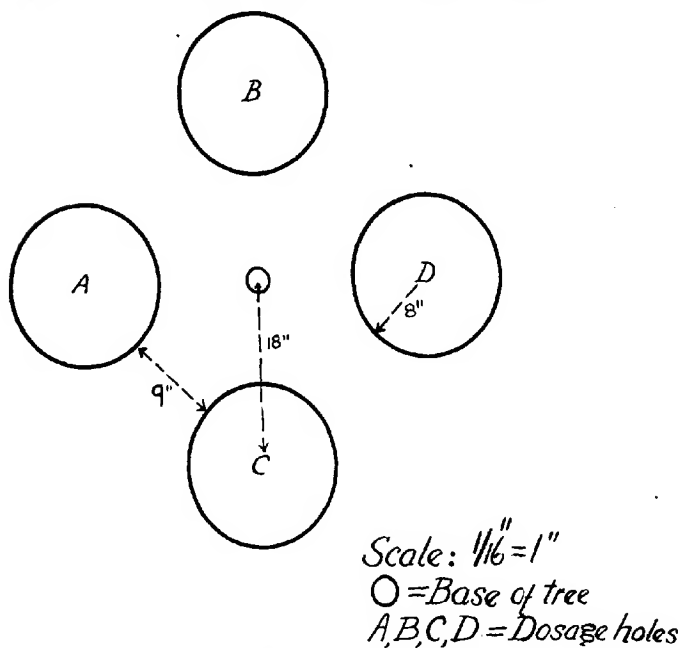
Fig. 8. Comparative Area of Root Injury

of both ages.   A consideration of figure 8, will explain this fact.   It can readily be seen that a given dose of carbon disulfide will produce as much injury to the roots in the vicinity of a dosage hole when used on a 5-year-old tree as when used on a 2-year-old tree.   An equal number of roots radiating from the base of the tree will be killed in one as in the other.   Furthermore, the older tree will be much slower in outgrowing the injury because the roots of the former, girdled near the base of the tree as a result of the carbon disulfide gas, are much longer than are the roots of the younger tree, and consequently less easily replaced.   In the case of the older tree the normal proportion of top

growth to root growth is shattered completely, while with a young tree this condition is more or less temporary.

Soil type. The soil type is a factor influencing the degree of injury only inasmuch as it affects the diffusion of the carbon disulfide gas in the soil. In a shale soil, for example, a dose of $\frac{3}{4}$ ounce will have a diffusion of 36 inches and the rootlets will be killed for this distance or a slightly greater distance. In a stiff clay soil, on the other hand, a dose of $\frac{3}{4}$ ounce will give but 15 to 18 inches diffusion, and the rootlets will be killed for a slightly greater distance. The area of rootlets killed may therefore be said to depend on the area of diffusion of the gas, which in turn varies with the soil type.

Soil moisture. In some cases, a lack of sufficient moisture in the soil will result in decreased diffusion and consequently decreased killing of the rootlets. In a very dry shale soil, for example, with 7.75 per cent moisture, a dose of $\frac{3}{4}$ ounce will give a diffusion of 18 inches and the rootlets will be killed for the same distance. Twice the area of diffusion and root killing takes place in moist shale soil.

Seasonal injury. The dosage tests outlined above indicate that the time of year in which trees are treated with carbon disulfide is not the controlling factor responsible for the injury resulting from the treatment, and that the roots of the apple are apparently at no time entirely resistant to the fumes of the carbon disulfide. It is evident that the degree of injury will depend upon the efficiency of the diffusion, and, regardless of the time of year, if the soil is in proper shape to allow for diffusion, injury will result to the root system located in the path of the diffusion.

However, the indirect effect of this root injury upon the portion of the tree above ground varies considerably with the season and consequent stage of seasonal tree growth. In this respect the most susceptible period at which trees can be treated is the time in the spring between the swelling of the buds and the appearance of the first leaves. A dose that will completely shatter the top of the tree at this stage will simply burn the tips when applied later in the summer or fall. However, the fundamental root injury is the same in both cases, and for this reason it is never a safe procedure to estimate injury by an examination of the top.

The comparative resistance of weak and strong trees. During the course of the work, many parallel tests were carried out with strong trees and weak trees. Injury resulting from the use of a given dose was about the same in both cases, but the strong trees outgrew the injury more rapidly.

The comparative resistance of the vine and apple tree to the fumes of carbon disulfide

An examination of the French-literature dealing with the control of the grape *phylloxera,* by the use of carbon disulfide discloses some interesting data regarding the resistance of the vine to this material when injected in the

soil. It is of especial interest when compared with the data, obtained during the course of this work, on the resistance of the apple to this same treatment.

Marion (5) discusses the action of the fumes of carbon disulfide on the grape as follows:

> The nature of the histologic lesions produced by the vapors of carbon disulfide on the root formation, as shown by the microscope explains the exterior phenomena which are manifested.
>
> The functions of the roots are suspended, the upward progress of the sap is arrested; and, as the transpiration continues from the green portion, the latter is enfeebled as a result. Because of this lack of water, the phenomenon of reduction of which the sap must furnish the elements, is interrupted and the functions of the chlorophyll are arrested and the foliage assumes a yellowish or reddish tint. If this state is prolonged the exhaustion of the plant will prove fatal; but the vapors of carbon disulfide disappear from the soil, the roots begin to absorb again, the sap reaches anew the green portion of the vine and the normal condition is reëstablished.

It is readily understood then, how watering can hasten this reestablishment, why the affected vines can manifest later a revival in their vegetation, and why the strong vines, of which the tissues are still filled with sap, can support, without grave inconvenience, this momentary suspension in the absorption of water, while those which the *phylloxera* have already injured, of which the tissues are already almost desiccated, succumb so easily to the presence of the vapors of carbon disulfide around the debris of their roots.

It would seem from the above statement, that when a strong, vigorous vine is treated during the growing season, with a quantity of carbon disulfide sufficient to rid it of the *phylloxera*, the vine will suffer a temporary distress due to interference with transpiration but will not experience a degree of rootlet injury sufficient to impair seriously or kill the vine. The dosage tests detailed above indicate the entirely different effect of carbon disulfide upon the apple. Here, a dose, no matter how small, produces a certain degree of injury to the roots and rootlets in the path of its diffusion, and the amount of injury caused by the employment of a dosage sufficient to rid the apple of the woolly aphis, prohibits the use of the treatment.

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THE EFFICIENCY OF THE TREATMENT

The use of carbon disulfide as a control measure does not result in 100 per cent efficiency. Aphids infesting roots, growing near the surface of the soil, or in decomposed trash at the surface of the soil, are prone to escape the action of the fumes. The few thus escaping serve as a source of reinfestation. However, were it not for the injury arising from the use of the material, young apple trees infested with woolly aphis could be maintained in a vigorous condition, with one annual treatment at the most, for the number of aphids would be so reduced as to render the injury caused by them negligible.

The efficiency of carbon disulfide as a control for the grape *phylloxera* is due to the fact that by means of its periodic use, the vines can be maintained in a vigorous condition, capable of producing a normal crop of grapes. This is rendered possible because the numbers of *phylloxera* are kept down to such a point that the injury produced by them is insignificant.

SUMMARY

The conditions which influence the diffusion of carbon disulfide in the soil are: first, the soil type; second, the soil moisture; and third, the depth and arrangement of the dosage holes. Of these three factors, the moisture content of the soil is the limiting one. Variations in atmospheric or soil temperature throughout the growing season apparently do not affect the attainment of maximum diffusion and for this reason it can be secured at any time during the growing season when soil-moisture conditions are favorable.

It was found impossible to apply carbon disulfide at any time during the year when soil conditions were favorable without causing injury to the apple tree. Direct injury resulted to the roots and indirect injury to the branches and foliage, apparently due to the interference with normal transpiration.

REFERENCES

(1) Association of Official Agricultural Chemists   1908   Official and provisional methods of analysis.   U. S. Dept. Agr. Bur. Chem. Bul. 107, p. 13–14.

(2) GASTINE, G., AND COUANON, G.   1884   Emploi du Sulfure de Carbone contre le Phylloxera.   Paris.

(3) LEACH, B. R.   1918   Experiments in the control of the root form of the woolly apple aphis.   U. S. Dept. Agr. Bul. 730, p. 29–40.

(4) MARION, A. F.   1877   Treatment des Vignes Phylloxerees par le Sulfure de Carbone.   Paris.

(5) MARION, A. F.   1877–78–79   Application du sulfure de carbone au traitement des vignes phyloxerees.   Compagnie des Chemins de Fer de Paris et à Lyon à la Mediterranée.

PLATE 1

Tube of wire screening, used in determining the behavior of carbon-disulfide gas in the soil.
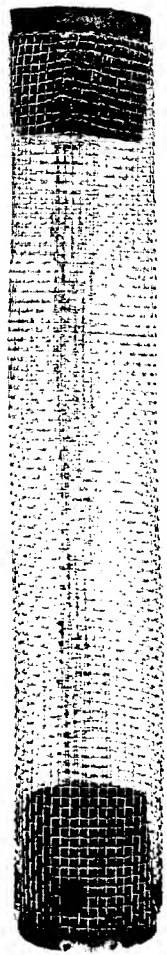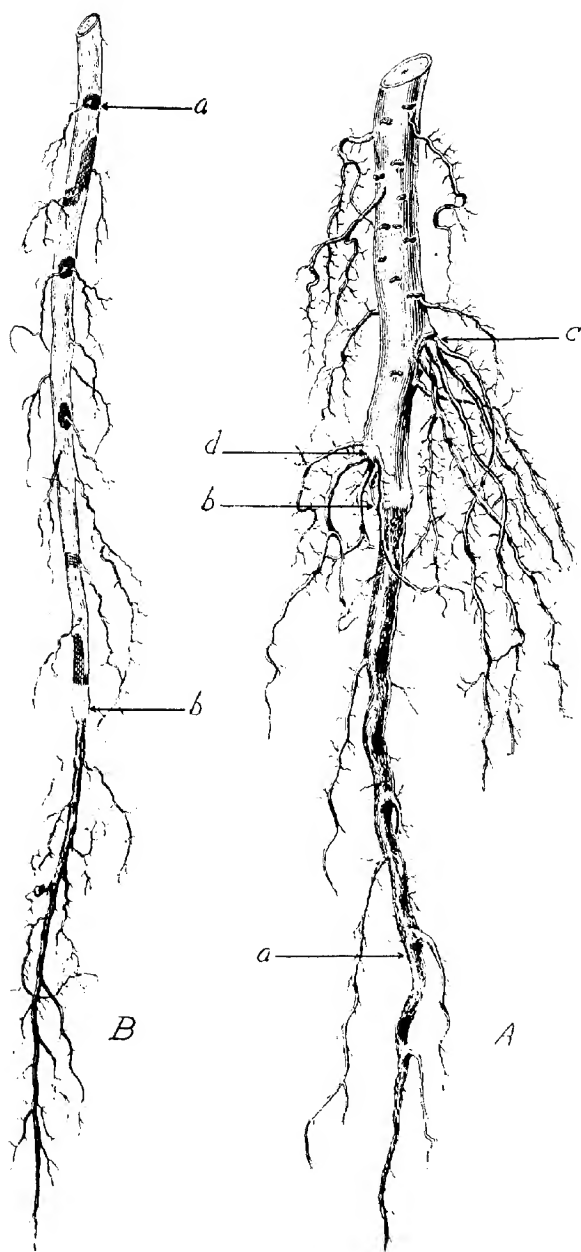
PEAT DEPOSITS IN THE UNITED STATES AND THEIR CLASSIFICATION

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AREA AND GENERAL DISTRIBUTION

The peat deposits in the United States of both fresh-water and salt-water origin have been variously estimated in regard to area, location, and character.

The report of the Commissioner of the General Land Office for 1907 gives 65,582,503 acres as the area of swamp land, claims for which had been approved and certified under various acts.

From Wright's results of a canvass made in the same year by the Office of Experiment Stations (21) it appears that alone in the eastern portion of the United States there are 77,000,000 acres of swamp and overflowed land.

In Senate Document No. 443 of the sixtieth Congress, first session, 1908, the estimate by Elliott is placed at 79,005,023 acres in the several states, exclusive of the coast lands which are overflowed by tidewater. They are listed as permanent swamp, wet grazing land, periodically overflowed land and periodically swampy land; this classification represents approximately the relative agricultural value of the land as affected by surface water conditions (20).

Davis (8) finds that the total area of peat land is nearly 140,000 square miles. Of this 8 per cent, or about 11,200 square miles, is assumed to be peat deposits of good quality, averaging at least 9 feet in thickness.

McCrory (12), summarizing the drainage movement in the United States, gives as the most reliable information obtainable concerning the undeveloped area of swamp and wet lands a total of 102,000,000 acres, of which 66,900,000 acres are swamp, 31,500,000 acres are periodically overflowed and 4,400,000 acres are tidal marsh.

According to Baker and Strong (1) only about 60,000,000 acres are swamp and other wet lands, and practically none of this land, which would make 1,000,000 farms of 60 acres each, is reported to be available for settlement at present.

More recently a report has been issued upon the unused lands in this country, as House Document No. 262, Sixty-sixth Congress (10). The document gives the possible projects which are available in the various states for development. The data include acreage of swamp land, cut-over land, wet grazing land,

overflow land, and periodical swamp land. In distribution, acreage and classi-fication House Document No. 262 of 1919 does not differ essentially from the report of 1908 by Elliott cited above.

From this brief summary it is obvious that the estimates of the actual areas of swamp and overflow lands are quite unlike; they differ still more widely when an attempt is made to distinguish the acreage which more properly constitutes workable peat deposits, and the percentage that is utilized at present for agricultural and other purposes in the different parts of the country.

As to distribution, the several authors agree more or less that the areas of peat accumulation may be assigned to two or three partly geographic regions which express the chief differences in climate, in surface vegetation, and in topography, especially those which are the results of glacial action. The deposits located in the northern region (I) may, therefore, be divided into those of the northeastern states (a), and the Lake states (b), while those in the southern or Atlantic coastal region (II) would include the Mississippi River bottoms and other river bottoms and terraces of the Coastal Plain of the South (c) and of the Gulf States with their adjoining territory (d). The known workable deposits in the Pacific coast region (III) occur in the tule lands of California and in the basins and rivers of Washington and Oregon.

The classification and acreage in table 1 is that of Elliott (20) and is repro-duced here in a form to indicate the three general regional divisions.

GENERAL CHARACTERISTICS OF PEAT DEPOSITS

For diagnostic and systematic purposes as well as for other lines of work in agriculture and in the industries a full knowledge of the acreage, location and of the development and structure of workable peat deposits will naturally be of the greatest importance. As the life histoιy of every deposit can now be studied without difficulty, provided the different layers of peat material are known, undoubtedly all our peat-land studies will gain in much needed conformity and accuracy.

Progress in peat investigations has been severely checked by the widespread use of such terms as muck, overflowed land, swampy land, wet land and others. The same error that has caused serious difficulties in the establishment of types of peat material has resulted in the lack of an adequate classification of peat deposits conforming with scientific viewpoints. Undoubtedly the adherence to composite terms unqualified in their meaning, is in part the reason why the structural features of peat deposits have been completely overlooked in this country and why thorough research in this direction has been delayed. A somewhat more critical attitude would long ago have revealed the façt that peat deposits are essentially a product of the movement of plant populations; that they have a characteristic development and struc-ture which is related to particular habitat conditions. This concept has been discussed in detail elsewhere. It will suffice to summarize here the several

TABLE 1

*Classification of unreclaimed swamp and overflowed land**

RE-GIONAL DIVI-SION	STATES	PERMANENT SWAMP	WET GRAZING LAND	PERIODICALLY OVERFLOWED	PERIODICALLY SWAMPY	TOTAL
		acres	*acres*	*acres*	*acres*	*acres*
IId	Alabama.............	900,000	59,200	520,000		1,479,200
IId	Arkansas.............	5,200,000	50,000	531,000	131,300	5,912,300
III	California.............	1,000,000	1,000,000	1,420,000		3,420,000
Ia	Connecticut..........		10,000	20,000		30,000
IIc	Delaware.............	50,000	50,000	27,000	200	127,200
IIc	Florida...............	18,000,000		1,000,000	800,000	19,800,000
IIc	Georgia	1,000,000		1,000,000	700,000	2,700,000
Ib	Illinois...............	25,000	500,000	400,000		925,000
Ib	Indiana	15,000	100,000	500,000	10,000	625,000
Ib	Iowa.................	300,000	200,000	350,000	80,500	930,500
	Kansas...............		59,380	300,000		359,380
	Kentucky............		100,000	300,000	44,600	444,600
IId	Louisiana.............	9,000,000		1,196,605		10,196,605
IIc	Maryland	100,000		92,000		192,000
Ia	Maine................	156,520				156,520
Ia	Massachusetts........	20,000		39,500		59,500
Ib	Michigan.............	2,000,000	947,439			2,947,439
Ib	Minnesota............	3,048,000	2,000,000		784,308	5,832,208
IId	Mississippi...........	3,000,000		2,760,200		5,760,200
	Missouri..............	1,000,000		1,439,600		2,439,600
	Nebraska.............		100,000	412,100		512,100
Ia	New Hampshire.......	5,000		7,700		12,700
Ia	New Jersey...........	326,400				326,400
Ia	New York............	100,000	100,000	329,100		529,100
IIc	North Carolina........	1,000,000	500,000	500,000	748,160	2,748,160
	North Dakota.........	50,000	50,000	50,000	50,000	200,000
Ib	Ohio.................			100,000	55,047	155,047
	Oklahoma			31,500		31,500
III	Oregon...............	254,000				254,000
Ib	Pennsylvania			50,000		50,000
Ia	Rhode Island			6,000	2,064	8,064
IIc	South Carolina........	1,500,000		622,120	1,000,000	3,122,120
	South Dakota.........	100,000		511,480		611,480
	Tennessee............	639,600				639,600
IId	Texas................	1,240,000	1,000,000			2,240,000
Ia	Vermont.............	15,000		8,000		23,000
IIc	Virginia..............	600,000		200,000		800,000
III	Washington..........	20,500				20,500
	West Virginia........			23,900		23,900
Ib	Wisconsin	2,000,000			360,000	2,360,000
	Total	52,665,020	6,826,019	14,747,805	4,766,179	79,005,023

* After Elliott, from Senate Document 443, 60th Congress, 1 Session 1908 the Abbreviations prefixed to the States are added by the writer to show the general regional divisions referred to in the text on p. 454.

main points in connection with their bearing upon the stratigraphic relations of peat deposits in the United States and in Europe.

An account of the general features of peat deposits and a definition of the term will naturally differ according to the point of view. On the economic side, the features of fundamental importance are those which qualify a deposit as a resource of raw materials of value for fuel, for distillation purposes, for manufacturing various finished products, as medium for beneficial bacterial activity, or for use in other specific ways. To the geologist, the most striking feature lies in the fact that a peat deposit may be regarded as a portion of the earth's surface-layers which represent an initial stage in the formation of coal beds and petroleum.

On the agricultural side, a peatland area leads to the consideration of its characteristics as an organic soil which furnishes support and nourishment to cultivated plants, but is generally unbalanced in the supply of inorganic plant-food constituents. The features of chief importance to agricultural aspects are special methods of drainage, fertilizing, cultivation and suitable cropping systems with which to maintain the fertility of organic soils and to make them increasingly serviceable to the community as a source of food for man and cattle. But all of these viewpoints may be summed up in the biological concept which regards peat deposits as formed by the successive growth of vegetation units, which now constitute accumulations of more or less disintegrated plant remains. The different layers of peat material were laid down in a definite manner according to imposed field conditions. The efficient use of peat deposits, whether as an available resource for food, power or finished products depends in large part upon the kind of organic material, the structure of the deposit, and the controlling factors in field conditions. The feature of noteworthy significance is that a peat deposit and its field conditions are to be regarded as correlated with each other in development and structure, in time and space relations.

In general, it would be advantageous to adopt the word moor as a synomym for peat deposit. Both terms should be limited to an accumulation of plant remains of at least 8 to 10 inches in thickness when compact and well shrunk. In this accumulation, the surface layer of living native vegetation and deposits containing more than 40 per cent of mineral matter should not be included. This restriction of the term would prove useful for purposes of mapping, and it would make for precision in surveys, in methods of practice, in instrumental investigation, in correlation studies, and in that large and increasing line of inquiry that depends on experimental work at field stations located in representative peatland areas.

CLASSIFICATION OF PEAT DEPOSITS

In evaluating peatland areas it is important to adopt a standard for classifying the deposits. The need of some method of classifying areas of peatland is as obvious as in the case of peat material resources.

Although classifications made by various investigators may differ widely because of the divergence in concept of the cause and factors controlling peat deposits and their uses, those differences become less pronounced as the development and structure of peat deposits becomes better known and as peatland problems are founded on scientific research in the field and in the technical laboratories, on which all applied work must ultimately be based.

While peat deposits may be grouped according to the practical "usage" point of view from which the subject is approached, that is, from the consideration of agriculture or the manufacturing industries, it becomes evident that a classification made for convenience or for reference to mode of utilization will be unsatisfactory in that it tends to inexactness and uncertainty. Unexpected difficulties in the control of the water-table due to springs, in the quantity of sulfur contamination, or from any other evidence brought out after more detailed field work or laboratory research may suddenly cause a deposit to lose much of its reputed practical value and place it in the non-productive class. On the other hand, improvements in methods of utilization may again change greatly the value of the practical classification. Some deposits may be used in several ways while in others the material has only a limited possibility. The various uses for which peat materials are best adapted have been discussed in detail in former publications (6, 7). The progress of peatland agriculture and the expansion of peat industries depend for their success upon the kind of raw material used, and hence due care must be observed in the selection of a workable deposit. In its larger aspect the criterion for a judicious choice should not only be fundamental, but also in line with the essential processes which initiate, continue or modify and finally terminate the development and structure of an accumulation of peat materials. It may be difficult for the layman to apply ecological and stratigraphic principles and to work out the basic factors of field conditions; it may be cumbersome for peatland operators to determine the relative importance of climatic, geologic, vegetational and topographic influences, especially where it is not readily evident whether the most striking cause may not be the less important and significant one in limiting the possibilities of a peat deposit. Nevertheless the view seems well supported from past failures that in the utilization of peat deposits for one or more practical purposes the field conditions and the layers of peat material are controlling factors in a profitable industry, and that the life history of the deposit should be carefully considered by prospective producers.

System of classifying peat deposits based on surface vegetation

Various comprehensive studies and classifications of peat deposits in America and elsewhere have been made according to the surface vegetation. There need be no objection to such terms as marsh, fen, bog, heath, shrub, forest, which represent a well marked physiognomy of vegetation. They are common names in many languages and the field conditions of each have a more or

less differentiating character, notably in the relation of the ground-water level to the surface of the soil. The possibility of using the native surface vegetation as a basis for differentiating between deposits of peat rests, however, entirely on the provision that the relation between the living vegetative cover, the character of the profile structure of the deposit, and the nature of the underlying mineral soil, is correctly interpreted. The degree of natural drainage which establishes itself in time on the surface of a deposit often determines the character of the vegetative cover. As a rule, deciduous trees such as maple, ash, elm and ·others are looked upon to indicate a better quality of deposit but exceptions are not uncommon where the material on which grow tamarack, spruce, or cedar would yield better results. The accumulation of vegetable material in the layer just below the surface vegetation is by far more frequently the decisive factor on reclaimed peat deposits, and hence of greater significance than the thin veneer of material formed from the plants on the surface of abandoned peat deposits or in consequence of fire or drainage.

There are few deposits which have not some kind of a structure formed during the various periods of time when the peat materials were deposited. In dealing with surface vegetation alone as an indicator, it would be impossible to determine the extent to which the vegetative cover is a characteristic of that structure or whether it was at all times successively the top layer at that point. A clearer concept will be gained in regard to the value of surface vegetation for distinction between peat deposits when accumulations of peat are considered in the light of their origin. Continuous deposition of a peat material from any type of surface vegetation would produce a deposit nearly uniform throughout, provided the field conditions remained constantly the same; the thickness of accumulation would be commensurate with the rate of deposition, the length of time and the nature of the plant remains. Variation in the composition or in the nature of the vegetation unit forming peat, or a change in field condition would bring about a commensurate change in the character of the deposit. The great changes in the stratigraphic features of peat deposits may safely be regarded as indicative of major changes in the surface vegetation at that particular time, and in the field conditions during that period. There are deposits in which the course of development has been a conversion of forest to marsh and finally to open water, and there are others the structure of which shows alternations between or different combinations in the superposition of several types of peat material. The occurrence of sedimentation, for example clay and sand from floods or from subsidence, as intercalations between layers of peat, serves to qualify very markedly any relationship between surface vegetation and the character of the deposit. It is necessary to bear in mind that by itself a surface vegetation of any unstable stage in the formation of peat deposits can not be regarded as significant in the determination of groups, classes, or other divisions of peatland areas. As a rule. the present plant populations at the surface, many of which have been described and classified elsewhere (4, 6) enter but little into the structure

of the larger portion of a deposit. The older or lower layers of peat deposits of the Scandinavian countries, of Holland, Germany, Switzerland, Finland and Russia, have a surprising number of features in common with some of the peat deposits in this country. With further accumulation of plant remains this feature becomes less marked; those of the regional distribution of plants and of climatic differences gradually increase in effectiveness. In surface vegetation of today many American and European deposits are quite unlike.

Topographic classification of peat deposits

There are peat deposits which are obviously associated with topographic conditions. They represent filled lakes and ponds, accumulations within and between moraines, on till plains and other flat areas and combinations of these types of land surface. However, in another paper dealing separately with representative groups of peat deposits, it will be possible to indicate that many of them are far from showing an exclusive relation to topographic conditions. These deposits comprise accumulations of plant remains in ancient drainage channels, glacial lake basins, old wave-built terraces or plains bordering the ocean and other important land forms which in all cases were free from water when marsh, forest and other vegetation units began to occupy these areas. They are among the chief sites of peat accumulations of the present time. Furthermore, the present streams and smaller drainage channels of peat lands are as a rule very young, having been established in most cases subsequent to the formation of the peat deposits. Most of the waters now flow on top of several layers of peat material and in places flow over deposits in which they are forming new and more or less distant channels. The imperfections of the surface drainage, shown by that large acreage of so-called swampy, overflowed, and wet grazing lands, is due in great measure to the comparative shortness of time since the establishment of the present surface streams and the retardation consequent on vegetation obstructing the run-off.

It is a striking fact that in the past far-reaching vegetation changes appear to have taken place over a great variety of land forms and, in many cases, without any marked correlation on the part of any factor except the water relation. The underlying causes for many vegetation changes even of today are found more readily in the successions of plant populations which operate within the limits of the controlling climatic factor of the region concerned. But the chief objection to a primary classification of peat deposits on topographic differences is that it obscures the structural development of a deposit and adds to the difficulty of distinguishing peatland areas which result from a rise in the water level through barrier formation, from flooding due to increased rainfall, from subsidence of a region or other disturbances. Moreover, certain soil conditions may arrest the succession of vegetation and keep it stationary at a point far short of the possible development within the topography of the area, while elsewhere the influence of plant migration and

of regional differences in vegetation increases in effectiveness. It appears probable and in fact seems a necessary consequence of the direct and inferential evidences obtained by Swedish investigators during the past decade that the character and direction of the structural formation of a peat deposit depends more upon climatic changes than upon anything else.

European system of classifying peat deposits

The classification of peat deposits which resulted from European investigations has reference to the sequence or superposition of certain types of peat material found in the moors. Three kinds of deposits or moors are recognized as main stages of a developmental series (22); low moor or flat moor (Niedermoor, Flachmoor, Verlandungsmoor) the vegetation units of which give rise to the marsh group of peat materials; transition moor or intermediate moor (Zwischenmoor, Ubergangsmoor) from which shrub and forest types of peat are derived, and highmoor (Hochmoor) which in the main is equivalent to sphagnum and heath types of peat. The tendency of some authors is to eliminate the transition moor since it appears to be less well characterized than the low moor and the high moor. The differences between the two latter in botanical composition of plant remains are well marked and they serve primarily for estimating the agricultural and industrial possibilities of these deposits. But the existence of upper and lower forest beds in peat land, found at the same horizon through deposits in widely separated regions, is of relatively great scientific importance. Shrub and forest types of peat indicate evidence, it is believed, for considerable changes in the environment during late glacial and postglacial times, and in the migration of vegetation. That peat deposits will yield abundant information on these points may be expected from the results already obtained in Europe by De Geer and Sernander (9), von Post (15), Samuelsson (17), Weber (22) and others.

The European system embodies the results of prolonged consideration and is well adapted to delineate the pronounced climatic influences of the northern part of that continent. But the continental system of classifying peat deposits is too limited for satisfactory discriminations in other countries. It is not suitable to peatland areas of other climates and to those deposits which upon examination show that other kinds of developmental and structural features are possible than those illustrated by the relative position of high-moor peat materials resting on low-moor types of plant remains. The striking cross-sections of American peat deposits such as the Kankakee marsh in Indiana, the Everglades of Florida, or the Dismal Swamp in Virginia and North Carolina are typical examples of what may be expected in the space and time relations of other kinds of peat deposits. They give a significant interest to investigations and to experimental work of peat deposits elsewhere which were formed under climatic influences quite unlike those of northern continental regions.

Classification of peat deposits based on chemical analyses

A distinction is often made between peat deposits on the basis of chemical differences, especially in lime content or in acid reaction. These and other chemical distinctions are unreliable when not related to type of peat material and they are inadequate to be used for purposes of classifying peat deposits. Chemical analyses are difficult to interpret and they are practically worthless when a peat deposit consists of a series of different layers of peat, and especially so when some of these are separated by intercalated mineral sediments, or contain siliceous, sulfureous or ferruginous material as contaminations or as depositions by plants and animals. Cases of that kind are numerous in deposits of peat near rivers and subject to overflow.

In the greater number of deposits a low content of lime or other mineral constitutents may be regarded as due partly to the underlying soil and geologic formation. A clay or marl substratum usually indicates fertilizer requirements quite different from sandy subsoils. Differences in quantity and in quality of salt content become observable as a rule after drainage, and under cropping systems which favor evaporation of the ground water from the surface layer of the organic soil. These conditions may affect all kinds of deposits alike when they are under cultivation and shrink. It is important to note that the influence of capillary action upon salt solutions, which Briggs and Lapham (2) have investigated, is related also to capillary limits in types of peat, and that studies on absorption and differential deposition, like those of Cameron (3) and of Patten and Waggaman (14), should be carried out on peatland areas before a chemical or a fertilizer analysis may be of value in differentiating between peat deposits. The fact should not be overlooked that layers of peat at different levels or the removal of surface material may give rise to entirely different chemical reactions.

The physical and chemical changes which take place in peat deposits are brought about to some extent by micro-organisms. Their presence in peat deposits has been demonstrated by a number of investigators (11). Some of the chemical reactions in bacterial culture studies with several types of peat have been reported in an earlier work (6) in connection also with the problem of bacterial by-products in peat deposits, contributing to the xeromorphy in the vegetation of the coal age (5). Results secured in Europe and in this country indicate that requirements of chemical fertilizers are often for the purpose of balancing products due to bacterial activity, for example, an excess of available nitrogen. Green plants on peat deposits utilize water and the carbon dioxide to form food, the starches, sugars and fats necessary to their nourishment. The mineral soil constituents are indispensable, but their amount is very small for any native vegetation unit growing on peat deposits and alone the inorganic salts are incapable of sustaining life in plants.

It is obvious, therefore, that a chemical classification of peat deposits based on initial tests or requirements must differ considerably from one made at a

later period and it is apparent also that it would be in general easier to remedy
the chemical deficiences than to change the nature and characteristic influ-
ence of the type of peat material or the structure of the deposit.

The stratigraphic system of classifying peat deposits

In a system of classifying peat deposits which is to conform with the devel-
opment and structure of such deposits, it is clear that the genesis and sequence
of peat materials must constitute the chief basis for grouping; it must furnish
basic criteria concerning the causes and methods by which the structural
features, changes and conditions have been produced or can be utilized. A
stratigraphic classification of peat deposits such as the writer proposes thus
includes more especially the two great primary divisions of water-laid and
land-laid peat deposits, the latter of which show as the noteworthy character-
istic the presence of roots in the mineral substratum. This system necessarily
includes and is based upon a botanical analysis of the peat materials them-
selves, of their sequence and relative position. It represents the historical
and genetic view in its broader sense, deals with the structural features which
deposits exhibit and, furthermore, takes careful account of the past physical
conditions of the environment. Nor does it leave out of consideration, the
various regional and climatic changes and their influence during the entire
period in which the deposits were formed. The botanical composition of
peat materials, their successional position in a deposit, the habitat conditions
which they reflect, all have their importance but their chief value lies in corre-
lations and in pointing out the basic process by which a peat deposit arises
and culminates, becomes stabilized or converted. A peat deposit undergoes
changes in structure until it comes into equilibrium with environmental con-
ditions and thereupon continues without further change in structure. When-
ever the conditions are changed climatically, physiographically, by migration
or alteration in plant and animal populations, a corresponding change of
structure results. But this change does not necessarily imply advance. Often
it is towards neither a progressive nor retrogressive structure, but a new
combination, a complex or mosaic which spreads upon and supplants the
earlier structural features. In the light of the stratigraphic results obtained
it should not be difficult to find out to what extent the life history of the various
deposits is essentially the same or presents striking differences. More com-
plete studies, of course, will contrast these differences, and they will soon
furnish a more correct insight. Those who are cognisant of the meaning of
these facts from an economic as well as a scientific point of view will be able
to realize how important and urgent is the need for looking more fully than
heretofore into the structural features of peat deposits and not only of investi-
gating the main types of peat material but also of obtaining a further measure
of technical and mechanical utilization of these stores of accumulated energy.

The fundamental problem in a stratigraphic or developmental classification
of peat deposits is, however, not alone the local sequence of types of peat

material, but also the equivalence of these types in different geographical regions. If principles and processes are of universal application, it is important to know to what extent the layers of one deposit may constitute a natural group strictly homologous in its age or time relation, with all the layers of a similar deposit elsewhere. The establishment of likenesses and differences among peat deposits, and of a time relationship between peat deposits of separate morainal systems and of different regions has not been as yet among the aims of American workers in peat problems. The work of foreign investigators, though familiar to the specialist, has generally escaped the attention of the student. Hence it has not been possible hitherto to contribute either toward establishing the identity and correspondence or to determine the exact time equivalence of peat deposits of great recessional moraines and between different countries. There appears to be little doubt that the identification of layers of peat material by means of the plant remains included in them will make feasible a grouping of deposits which formed during a common substage in the glacial period, and which may thus be referred to or based on a general age and time scale.

The period of the Wisconsin stage of glaciation was not a single advance and melting of the ice sheet but was somewhat complex. The early stage of the retreat of the ice sheet, which was marked by the Shelbyville and the Bloomington morainic systems, appears to have been followed by a readvance of the ice and by a shifting of the line of flow as the ice again spread out over the surface of the land. This readvance has been designated as the later Wisconsin stage. The limit of the readvance is marked by the Valparaiso-Kalamazoo-Mississinewa morainic systems.

The stratigraphic study and the mapping of peat deposits distributed within the series of important positions of the ice border as the result of the melting of the glaciers, may furnish valuable data for the study of past climatic changes. The various morainic groups, such as the Shelbyville, the Bloomington, the Valparaiso-Kalamazoo-Mississinewa, the Lake Border-Defiance, and the Port Huron morainic system represent climatic pulsations. They mark halting places during which the climate ceased to become milder and either remained nearly uniform for a while or else reverted somewhat toward the conditions which induce glaciation. It is obvious, therefore, that the sequence of peat materials in the deposits of these main systems may furnish the data for characterizing the climate of the earlier and the later Wisconsin stages and of the successively less extensive portions of the ice front. Perhaps a further classification of the peat deposits of the United States into two main subdivisions—those beyond the invasion of the late Pleistocene ice age and those within the glaciated area of the country would prove more practical. The minor subdivisions, especially of the deposits relating to the successive positions of the ice border, might give a record of striking individuality. They outline areas over which may exist similar advantageous types of peat, and they may give much information regarding suitable deposits for experimental work in agricultural and industrial problems.

Many of the European workers refer the stratification of a peat deposit not to successive changes in the level of ground water but correlate the sequence of peat materials with alternating wet and dry periods which accompanied changes in climatic conditions. It will now be possible to extend the European investigations dealing with climatic changes to the glacial regions of North America and to show whether or not glaciations have been contemporaneous, whether they depended upon general or local causes, and whether plant populations have immigrated and were affected by alternating dry and humid periods. But until the plant remains in peat deposits are more extensively studied microscopically and along lines of modern investigations, as those of Lennart von Post (16) and his collaborators, similarity of sequence and resemblance of types of peat material will be taken as the criterion. The procedure may lead to many erroneous correlations. Correspondence in succession of peat materials cannot always be regarded to have significance in regard to time equivalence as well, for example, in those cases where the layer of peat of the same botanical character is not necessarily of the same age, or where deposits of the same geologic period may contain plant remains not at all of the same botanical composition.

It is thus seen that a classification based on stratification soon must take on a chronologic aspect, especially as workers in peat problems begin to perceive that only a limited number of peat materials may be formed during the lifetime of a deposit and that in some areas the strata are often separated from those above them by a sharp line of demarkation. For the detailed study of American peat deposits correlation by plant remains is undoubtedly the most reliable working method and to identify the chief groups of American types of peat with similar types in other countries may be, in many cases, not far from wrong. There is little doubt that the morphological basis of classifying peat deposits will gain in value when established on renewed and thorough microscopic study and methods such as those used, for example, by the "Upsala School" (9, 13, 16, 18, 19). It is equally evident that the study of American peat deposits must be pursued independently if the growing demands of peatland industries and agriculture are to receive adequate scientific coöperation.

REFERENCES

(1) BAKER, O. E., AND STRONG, H. M. 1918 Arable land in the United States. *In* U. S. Dept. Agr. Yearb. 1918, p. 433–441.

(2) BRIGGS, L. J., AND LAPHAM, M. H. 1902 Capillary studies. U. S. Dept. Agr. Bur. Soils Bul. 19.

(3) CAMERON, F. K. 1906 Calcium sulphate in aqueous solutions. U. S. Dept. Agr. Bur. Soils Bul. 33.

(4) CLEMENTS, F. E. 1916 Plant succession: An analysis of the development of vegetation. Carnegie Inst. Washington Pub. 242.

(5) DACHNOWSKI, A. P. 1911 The problem of xeromorphy in the vegetation of the carboniferous period. *In* Amer. Jour. Sci., v. 32, p. 33–39.

(6) DACHNOWSKI, A. P. 1912 Peat deposits of Ohio. Ohio Geol. Survey Bul. 16, p. 4. (In cooperation with U. S. Bur. Mines.)

(7) DACHNOWSKI, A. P. 1919 Quality and value of important types of peat material. U. S. Dept. Agr. Bur. Plant Indus. Bul. 802.

(8) DAVIS, C. A. 1912 The uses of peat for fuel and other purposes. U. S. Bur. Mines Bul. 16.

(9) DeGEER, G., AND SERNANDER, R. 1908 On the evidences of late quaternary changes of climate in Scandinavia. *In* Geol. Förening. Förhandl., v. 30, p. 457–478.

(10) Development of unused lands. House Doc. No. 262, 66th Congress, 1st Session, 1919.

(11) LÖHNIS, F. 1910 Handbuch der landwirtschaftlichen Bakteriologie. Berlin.

(12) McCRORY, S. H. 1918 The drainage movement in the United States. *In* U. S. Dept. Agr. Yearb. 1918, p. 137–144.

(13) ODÉN, S. 1920 Bodenkundliche Forschungen an dem chemischen Universitäts-Laboratorium der Universitat Uppsala, 1914–1919. *In* Internat. Mitt. Bodenk., Bd. 9, p. 391–418.

(14) PATTON, H. E., AND WAGGEMAN, W. H. 1906 Absorption by soils. U. S. Dept. Agr. Bur. Soils Bul. 52.

(15) POST, L. VON 1909 Stratigraphische Studien über einige Torfmoore in Närke. *In* Geol. Förening. Förhandl., Bd. 31, p. 629–706.

(16) POST, L. VON 1916 Skogsträdpollen i sydsvenska torfmosslagerföljder. *In* Geol. Förening. Förhandl., v. 38, p. 384–390. Complete report *in* Förhandl. Skand. Naturf. Mote, p. 433–465. 1918.

(17) SAMUELSSON, G. 1910 Scottish peat mosses: A contribution to the knowledge of the late-quaternary vegetation and climate of northwestern Europe. Bul. Geol. Inst. Univ. Upsala, v. 10, p. 197–260.

(18) SANDEGREN, R. 1916 Hornborgasjön en monografisk framställning av dess postglaciala utvecklings-historia. *In* Sveriges Geol. Undersökn., Ser. Ca. No. 14.

(19) SERNANDER, R. 1908 Om ancylustidens människa och tall-perioden i södra Skandinavien. *In* Geol. Förening. Förhandl., v. 30, p. 388–398.

(20) Swamp lands of the United States. Senate Doc. No. 443, 60th Congress, 1st session, 1908.

(21) WRIGHT, J. O. 1907 Swamp and overflowed lands in the United States. U. S. Dept. Agr. Off. Exp. Sta. Cir. 76.

(22) WEBER, C. A. 1910 Was lehrt der Aufbau der Moore Norddeutschlands uber den Wechsel des Klimas in postglazialer Zeit? *In* Ztschr. Deut. Geol. Gesell., Bd. 62 (1911). Heft. 2, p. 143–162.

THE DETERMINATION OF HYDROGEN-ION CONCENTRATION BY THE COLORIMETRIC METHOD AND AN APPARATUS FOR RAPID AND ACCURATE WORK[1]

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During the progress of work under investigation for the last two years, it has been necessary to make a large number of determinations of the hydrogen-ion concentration of various solutions. For this work the indicators proposed by Clark and Lubs (2) were used and for the early part of the work their buffer solutions also were employed. Comparisons of colors were at first made in comparison blocks essentially like those of Hurwitz, Meyer and Ostenberg (5) and described by Clark and Lubs (2), but later an entirely new apparatus was devised which had the advantage of allowing more accurate comparisons and of permitting the determinations to be made more rapidly. This new apparatus, which is here described, has given excellent satisfaction for more than a year in the determination of hydrogen-ion concentrations ranging in pH values from 2.0 to 9.0 when it has been necessary, at times, to make as many as 100 determinations a day in addition to other regular laboratory work.

The preparation of the buffer solutions of Clark and Lubs (2) required so much time and care that it appeared worth while to adapt the apparatus for use with the more easily prepared double-tube standards suggested by Barnett and Chapman (1) and later improved by Gillespie (4). Some trials were conducted upon methods of preparing the double-tube standards and the final results of the observations made also are given.

The essential part of the new apparatus is the eyepiece which, in this case, was removed from an old colorimeter. It is shown diagrammatically and in actual size in figure 1, and photographically in plate 1, figures 1 and 2.

The frame for supporting the eyepiece was so made that wooden blocks holding the color tubes would slide in it before the eyepiece bringing the color tubes successively into view. Besides making the use of the tubes more convenient and rapid, the blocks serve to eliminate from the eyepiece, light coming from any source other than through the color tubes. One block holding the tubes of standard colors with known pH values is passed before one section of the eyepiece while before the other section of the eyepiece is passed another

block holding tubes containing the solutions of unknown pH values. When the pH value of one unknown has been determined, the next unknown may be passed quickly before the eyepiece and the block of standards moved to the right or to the left until a color is found which corresponds to the unknown and the pH value at once read off from a number on the block showing the pH value of the standard color tube before the eyepiece. The wooden blocks may be of any desired length, but for the tubes of standard colors it is most convenient to use a block long enough to accommodate at least a complete set of color standards for a single indicator.

Tubes best suited for use in this apparatus are screw cap homeopathic vials about 15 mm. in outside diameter and 46 mm. high. These are most conveniently used without the screw caps for the solutions of unknown pH values, but the standard colors may be kept and used for several days if the caps are partly filled with melted paraffin and screwed tightly onto the bottles after the paraffin has hardened.

If double-tube standards are to be used or if the single-tube standards are to be used with unknown solutions which are turbid, it is necessary to have blocks which are so made that they will accommodate two rows of tubes. One such block is shown in plate 1, figure 2. When the narrower blocks are used in a frame made to accommodate the wider blocks, it is well to use with them blocks which will shut out side light and admit only the light which comes through a ground-glass plate on the back side of the frame. If the double-tube standards are to be used while the pH values of turbid solutions are being determined, the frame will need to be made to accommodate blocks holding three rows of tubes, or one tube can be inserted into the framework back of each of the double-tube blocks.

The convenience of the apparatus, the rapidity with which it may be used and its adaptability are readily seen and further description, other than that accompanying the figures, is unnecessary.

Gillespie (4) tested out the behavior of several of the indicators first proposed by Clark and Lubs and by means of the hydrogen electrode found, as had previously been supposed, that at least several of them actually do follow the equation for the dissociation of a monobasic acid or of a monoacid base. The calculations for table 1 were made according to this equation which is:

$$a = \frac{k}{k + (H)},$$

in which a = the percentage dissociation (or per cent of indicator in the form giving the color produced by the dissociation products), (H) = hydrogen-ion concentration and k = a constant.

If the constant k in this equation is known, we may calculate what per cent of indicator used is in the form to give the acid color and also the amount in the form to give the alkaline color at any given hydrogen-ion concentration.
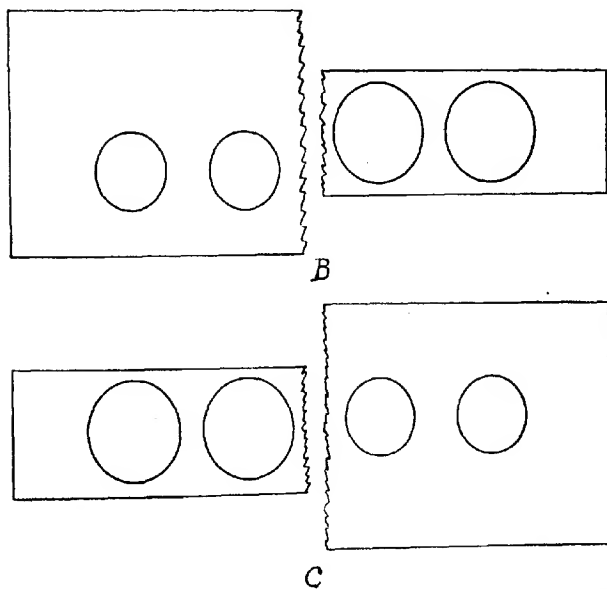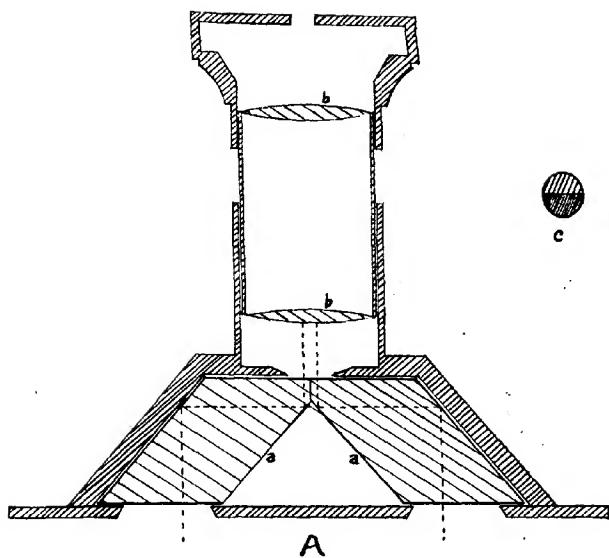
FIG. 1. EYEPIECE AND TUBE HOLDERS OF THE APPARATUS FOR COLORIMETRIC DETERMINA-
TION OF HYDROGEN-ION CONCENTRATION

A. Eyepiece—*aa*, glass prisms; *bb*, lenses; *c*, visible color disk.
B. Tube holder for upper opening of eyepiece when eyepiece is in operating position.
C. Tube holder for lower opening of eyepiece.

Clark and Lubs (2) have given the values of k for a number of indicators determined according to their method of finding the "half transformation points," the hydrogen-ion concentration corresponding to each of these points being the same in numerical value as that of the constant k for the indicator in question. Gillespie also has made some determinations of the constants by means of the hydrogen electrode. Using these constants as they have been determined, we may calculate values for a in the above formula which will correspond to any chosen number of hydrogen-ion concentration values. These calculated values may then be used to plot a curve which will show the percentage of indicator in the form giving the acid color at any hydrogen-ion concentration. Such curves for several indictors are given in the article by Clark and Lubs (2) and also in a more recent article by Lubs (6). It is not necessary, however, to have a curve for each indicator. It is necessary only to place the pH value which corresponds to the "half transformation point" of each indicator opposite the midpoint of the curve and reverse the direction of increasing pH values, for those indicators which behave as bases, from the direction given for indicators behaving as acids. How this may be done is more easily understood by referring to figure 2. This graph shows not only the per cent of indicator which is in the forms giving the acid and the alkaline colors when single-tube standards are used, but also the per cent of total indicator in both tubes of each pair that should be placed into each of the tubes when double-tube standards are used and equal amounts of solution plus indicator are placed into each tube.

Other indicators than those referred to below the graph, provided their dissociation follows the same equation, may be added to the list by simply finding the pH value corresponding to the "half transformation point" and placing it opposite the midpoint of the curve, then arranging the other pH values according to the behavior of the indicator as an acid or as a base.

Since the method of using the double-tube standards has been described both by Barnett and Chapman (1) and by Gillespie (4), it will not be necessary to repeat it here. It seems advisable, however, to show how a series of double-tube standards may be made more complete and to give a table which will assist in their preparation.

For determining accurately the per cent of total indicator to be placed in each of the tubes, the graph in figure 2 may be drawn to a larger scale or instead, a table may be prepared by means of the equation already given and made as complete as desired. Numbers on the accompanying graph show the percentages of indicator in the form giving the acid or alkaline color for pH values as close together as 0.1, which is as close as pH determinations can be accurately made colorimetrically in most cases. The actual amounts of indicator and the necessary dilutions to be used with the double-tube standards are shown in table 1, which shows variations of 1.0 per cent of indicator in the form giving the acid or the alkaline color when the tubes are viewed as they are when used in the apparatus here described. The percent-
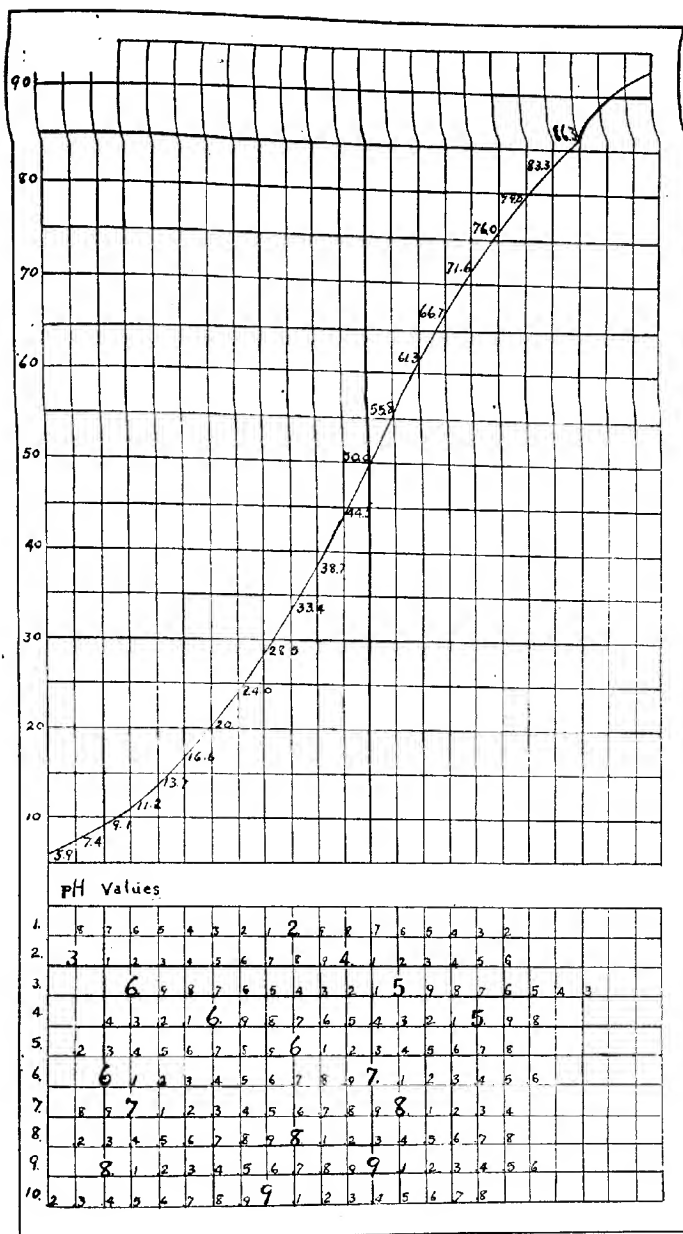
FIG. 2. GRAPH GIVING PERCENTAGE DISSOCIATION OF INDICATORS FOR DIFFERENT pH VALUES REPRESENTING PER CENT INDICATOR IN ACID COLOR FORM FOR INDICATORS NUMBERED 1, 3 AND 4 WHICH BEHAVE AS MONOACID BASES AND PER CENT INDICATOR IN ALKALINE COLOR FORM FOR INDICATORS NUMBERED 2, 5, 6, 7, 8, 9 AND 10 WHICH BEHAVE AS MONOBASIC ACIDS

The numbers at the left below the graph refer to the indicators as follows:

1. Thymol blue (acid range)	4. Propyl red	7. Phenol red
2. Brom-phenol blue	5. Brom-cresol purple	8. Cresol red
3. Methyl red	6. Brom-thymol blue	9. Thymol blue (alkaline range)
	10. Cresol phthalein	

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ages of indicator in the acid form and in the alkaline form for the different pH values chosen are given in the first two columns of the table. The two tubes of the double-tube standard may be prepared by measuring into them these percentages of the total amount of indicator used in both tubes, and diluting both to the same volume, one tube with a solution giving the acid color of the indicator, the other with a solution giving the alkaline color. It is evident that the same results may be obtained by using any amounts of indicator in either tube provided the proper dilution is made to give the same concentration of indicator as is obtained by the above procedure. Since it is difficult to measure the small amounts of indicator necessary in following the former procedure, requiring the use of several dilutions of each indicator, the same result has been obtained by adding to each tube an amount of indicator which is easily measured and obtaining the proper dilution by varying the amounts of the solutions with which they are diluted. The amounts of indicator given in the third and fifth columns of table 1 are easily measurable quantities arbitrarily chosen and the necessary amounts of acid and of alkali solutions to give the different percentage concentrations of indicator were then calculated according to the following formula:

$$x = \frac{a\left(b - c\,\dfrac{d}{100}\right)}{c\,\dfrac{d}{100}},$$

which may be simplified to the form:

$$x = \left(\frac{b}{c}\,\frac{100a}{d}\right) - a,$$

in which x = cubic centimeters of solution to be used in diluting the chosen amount (cc.) of indicator (d); a = cubic centimeters of indicator arbitrarily chosen for use in the tube under consideration (see third and fifth columns of table 1); b/c = ratio of solution plus indicator in either tube to total indicator in an equal volume of the mixture from each tube; d = percentage which the indicator in the tube under consideration represents of the total indicator in a like volume of the mixture from each tube (see first and second columns of table 1).

The ratio b/c may be varied according to the strength of indicator solution to be used and the depth of color desired in the tubes for comparison. In the calculations for table 1, b is given the value 2.2 and c, the value 0.2. Substituting these values, the formula as used becomes:

$$x = \frac{1100a}{d} - a.$$

Since calculations according to this formula are made on the basis that 2.2 cc. from the acid tube and 2.2 cc. from the alkaline tube contain a total of 0.2 cc. of indicator, the color of the observed light from double-tube standards prepared according to table 1, has been produced by as much total indicator as has the color of the light from the tube of an unknown solution, the pH value of which is to be determined, when 0.2 cc. of indicator has been added to 2 cc. of the unknown solution. For this reason, when standards made according to table 2 are used, the tubes of unknown should always be made up for comparison by adding 0.2 cc. of indicator to each 2 cc. of the unknown in tubes of the same diameter as the tubes used for the standards.

By means of the graph (fig. 2) and table 2 a double-tube color standard for any pH value may be easily and quickly prepared. For example, if a double-tube standard for the pH value 5.7 is desired, the graph shows that either methyl red or brom cresol purple may be used, 20.1 per cent of the former being in the form which gives the acid color of the indicator and 20.1 per cent of the latter being in the form which gives the alkaline color of the indicator. For practical work the nearest whole per cent is close enough, or, with methyl red, 20 per cent in the acid form and 80 per cent in the alkaline form. By referring to table 1 it is found that the proper dilutions for these percentages are obtained when, in one tube 0.1 cc. of the indicator is diluted with 5.4 cc. of the solution which brings out the required acid color, and in the other tube 0.2 cc. of the indicator is diluted with 2.55 cc. of a solution which brings out the required alkaline color.

Gillespie (4) brings out the alkaline color of the indicators by adding one to two drops of a 0.2 per cent solution of sodium hydroxide to each tube, and the acid color by means of 0.05 N hydrochloric acid or 2 per cent acid potassium phosphate (KH_2PO_4), from one drop to 1 cc. being added to each tube. The total volume in each tube is then made up to 5 cc. with water. According to the method here described, the acid and the alkaline colors of the indicators are brought out by the solutions with which the indicators are diluted. For this reason the solutions should have approximately definite hydrogen-ion concentrations. It is not necessary that these solutions contain strong buffers, but it is well that they should so that their hydrogen-ion concentrations may remain constant on standing.

With the indicators of Clark and Lubs giving a range of pH values from 1.0 to 10.0, it is best to have five solutions of different hydrogen-ion concentration values for making the dilutions. These should have approximately the following pH values and may be made according to the following suggestions.

1. 0.5 N hydrochloric acid having a hydrogen-ion concentration near 1.5 N (3, p. 15).

2. Solution with a hydrogen-ion concentration near $1 \times 10^{-1.6}$ containing 3.728 gm. of potassium chloride and 131.5 cc. of 0.2 N hydrochloric acid per liter (2).

TABLE 1

Amounts of indicator and of acid and alkaline solutions to be used in making double-tube color standards varying by increments of 1 per cent of indicator in either color form (pH values corresponding to the different percentages may be taken from the graph, figure 4)

INDICATOR IN ACID COLOR FORM	INDICATOR IN ALKALINE COLOR FORM	MAKE ACID COLOR WITH		MAKE ALKALINE COLOR WITH	
		Indicator	Acid solution	Indicator	Alkaline solution
per cent[*]	*per cent*[*]	*cc.*	*cc.*	*cc.*	*cc.*
5	95	0.05	10.95	0.20	2.12
6	94·	0.05	9.12	0.20	2.14
7	93	0.05	7.81	0.20	2.17
8	92	0.05	6.83	0.20	2.19
9	91	0.05	6.06	0.20	2.22
10	90	0.05	5.45	0.20	2.25
11	89	0.05	4.95	0.20	2.27
12	88	0.05	4.53	0.20	2.30
13	87	0.05	4.18	0.20	2.33
14	86	0.05	3.88	0.20	2.36
15	85	0.05	3.62	0.20	2.39
16	84	0.05	3.39	0.20	2.42
17	83	0.05	3.17	0.20	2.45
18	82	0.05	3.01	0.20	2.48
19	81	0.05	2.84	0.20	2.52
20	80	0.10	5.40	0.20	2.55
21	79	0.10	5.14	0.20	2.58
22	78	0.10	4.90	0.20	2.62
23	77	0.10	4.68	0.20	2.66
24	76	0.10	4.48	0.15	2.02
25	75	0.10	4.30	0.15	2.05
26	74	0.10	4.13	0.15	2.08
27	73	0.10	3.97	0.15	2.11
28	72	0.10	3.83	0.15	2.14
29	71	0.10	3.69	0.15	2.17
30	70	0.10	3.57	0.15	2.21
31	69	0.10	3.45	0.15	2.24
32	68	0.10	3.34	0.15	2.28
33	67	0.10	3.23	0.15	2.31
34	66	0.10	3.14	0.15	2.35
35	65	0.10	3.05	0.15	2.39
36	64	0.10	2.96	0.15	2.43
37	63	0.10	2.87	0.15	2.47
38	62	0.10	2.80	0.15	2.51
39	61	0.10	2.72	0.15	2.55
40	60	0.10	2.65	0.15	2.60
41	59	0.10	2.58	0.15	2.65
42	58	0.10	2.52	0.15	2.69
43	57	0.10	2.46	0.15	2.74
44	56	0.10	2.40	0.15	2.80
45	55	0.10	2.34	0.15	2.85
46	54	0.10	2.29	0.15	2.91
47	53	0.10	2.24	0.15	2.96

TABLE 1—*Continued*

INDICATOR IN ACID COLOR FORM	INDICATOR IN ALKALINE COLOR FORM	MAKE ACID COLOR WITH		MAKE ALKALINE COLOR WITH	
		Indicator	Acid solution	Indicator	Alkaline solution
*per cent**	*per cent**	*cc.*	*cc.*	*cc.*	*cc.*
48	52	0.10	2.19	0.15	3.02
49	51	0.10	2.14	0.15	3.09
50	50	0.10	2.10	0.10	2.10
51	49	0.15	3.09	0.10	2.14
52	48	0.15	3.02	0.10	2.19
53	47	0.15	2.96	0.10	2.24
54	46	0.15	2.91	0.10	2.29
55	45	0.15	2.85	0.10	2.34
56	44	0.15	2.80	0.10	2.40
57	43	0.15	2.74	0.10	2.46
58	42	0.15	2.69	0.10	2.52
59	41	0.15	2.65	0.10	2.58
60	40	0.15	2.60	0.10	2.65
61	39	0.15	2.55	0.10	2.72
62	38	0.15	2.51	0.10	2.80
63	37	0.15	2.47	0.10	2.87
64	36	0.15	2.43	0.10	2.96
65	35	0.15	2.39	0.10	3.05
66	34	0.15	2.35	0.10	3.14
67	33	0.15	2.31	0.10	3.23
68	32	0.15	2.28	0.10	3.34
69	31	0.15	2.24	0.10	3.45
70	30	0.15	2.21	0.10	3.57
71	29	0.15	2.17	0.10	3.69
72	28	0.15	2.14	0.10	3.83
73	27	0.15	2.11	0.10	3.97
74	26	0.15	2.08	0.10	4.13
75	25	0.15	2.05	0.10	4.30
76	24	0.15	2.02	0.10	4.48
77	23	0.20	2.66	0.10	4.68
78	22	0.20	2.62	0.10	4.90
79	21	0.20	2.58	0.10	5.14
80	20	0.20	2.55	0.10	5.40
81	19	0.20	2.52	0.05	2.84
82	18	0.20	2.48	0.05	3.01
83	17	0.20	2.45	0.05	3.17
84	16	0.20	2.42	0.05	3.39
85	15	0.20	2.39	0.05	3.62
86	14	0.20	2.36	0.05	3.88
87	13	0.20	2.33	0.05	4.18
88	12	0.20	2.30	0.05	4.53
89	11	0.20	2.27	0.05	4.95
90	10	0.20	2.25	0.05	5.45
91	9	0.20	2.22	0.05	6.06
92	8	0.20	2.19	0.05	6.83
93	7	0.20	2.17	0.05	7.81
94	6	0.20	2.14	0.05	9.12
95	5	0.20	2.12	0.05	10.95

* On the basis that 100 per cent is the total amount of indicator in equal volumes of solution plus indicator from each tube.

3. Solution with a hydrogen-ion concentration near $1 \times 10^{-5.6}$ containing 10.207 gm. of acid potassium phthalate and 199.25 cc. of N/5 sodium hydroxide per liter (2).

4. Solution with a hydrogen-ion concentration near 1×10^{-10} containing 3.1012 gm. of boric acid, 3.728 gm. of potassium chloride and 219.5 cc. of 0.2 N sodium hydroxide per liter (2).

5. 0.01 N potassium hydroxide having a hydrogen-ion concentration near 1×10^{-12} (3, p. 15).

TABLE 2

The hydrogen-ion concentration of solutions used to bring out the acid colors and the alkaline colors of the indicators

INDICATOR	H-ION CONCENTRATION OF THE SOLUTION				
	1.5 N	$1 \times 10^{-1.6}$	$1 \times 10^{-5.6}$	1×10^{-10}	1×10^{-12}
Thymol blue (acid range) pH 1.0–2.8	Acid color		Alkaline color		
Brom-phenol blue pH 3.0–4.6		Acid color	Alkaline color		
Methyl-red pH 4.4–6.0	Acid color	Acid color		Alkaline color	Alkaline color
Propyl-red pH 4.8–6.4		Acid color		Alkaline color	Alkaline color
Brom-cresol purple pH 5.2–6.8		Acid color		Alkaline color	
Brom-thymol blue pH 6.0–7.6		Acid color		Alkaline color	
Phenol red pH 6.8–8.4			Acid color	Alkaline color	Alkaline color
Cresol red pH 7.2–8.8			Acid color	Alkaline color	Alkaline color
Thymol blue (alkaline range) pH 8.0–9.6			Acid color		Alkaline color
Cresol phthalein pH 8.2–9.8			Acid color		Alkaline color

Since exact hydrogen-ion concentration values are not necessary in these solutions, ordinary C. P. chemicals may be used without additional purification.

Table 2 shows which of the solutions should be used in bringing out the acid and the alkaline colors of each indicator. It will be seen from this table that, between the pH values 3.0 and 8.8, only three solutions are necessary. For convenience and rapidity the solutions should be kept in bottles to which automatic burettes are attached for measuring out the desired quantities.

Apparatus for determining the hydrogen-ion concentration of solutions by means of the hydrogen electrode was not available and for this reason absolute accuracy is not claimed for pH values determined as here outlined. They should be accurate, however, for those indicators found by Gillespie (4) to follow the dissociation curve of a monobasic acid or a monoacid base. Whether

the pH values follow this curve accurately or not, the method furnishes an easy and rapid means of duplicating a given color.

If the use of single-tube color standards is preferred, these may be reproduced, when once they have changed color, by adding to the single-tube buffer solution, a solution of slightly higher or slightly lower hydrogen-ion concentration, as necessary, until the desired pH value is obtained, as determined by comparison with the double-tube standard. If this is contemplated and the single-tube buffer solution is to be taken as the standard, the single-tube colors and the double-tube colors should be matched when the single-tube buffer solutions have been freshly prepared, so that the proper double-tube standard may be used for comparison later.

REFERENCES

(1) BARNETT, G. D., AND CHAPMAN, H. S. 1918 Colorimetric determination of bacteriologic mediums and other fluids. *In* Jour. Amer. Med. Assoc., v. 70, p. 1062–1063.

(2) CLARK, W. M., AND LUBS, H. A. 1917 The colorimetric determination of hydrogen-ion concentration and its application to bacteriology. *In* Jour. Bact., v. 2, no. 1, p. 1–34; no. 2, p. 109–136; no. 3, p. 191–236.

(3) FRIEDENTHAL, H. 1904 Die Bestimmung der Reaction einer Flüssigkeit mit Hilfe von Indikatoren. *In* Ztschr. Electrochem. Angew. Phys. Chem., Bd. 10, p. 113–119.

(4) GILLESPIE, LOUIS J. 1920 Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with special reference to soils. *In* Soil Sci., v. 9, no. 2, p. 115–136.

(5) HURWITZ, S. H., MEYER, K. F., AND OSTENBERG, Z. 1915 On a colorimetric method of adjusting bacteriological culture media to any optimum hydrogen ion concentration. *In* Proc. Soc. Exp. Biol. Med., v. 13, p. 24; *also in* Johns Hopkins Hospital Bul., v. 27, p. 16.

(6) LUBS, H. A. 1920 Indicators and their industrial application. *In* Jour. Indus. Engin. Chem., v. 12, no. 3, p. 273–274.

PLATE 1

FIG. 1. APPARATUS FOR DETERMINING HYDROGEN-ION CONCENTRATION COLORIMETRICALLY

The eyepiece is raised from its position when in use to show the circular openings through which the color tubes are observed. Both tube holders may be slid in either direction so as to bring the different tubes before the eyepiece. The lower tube-holder is for the standard color tubes and the labels on the holder are so numbered that the first visible number at the right of the eyepiece indicates directly the pH value of the standard color tube before the eyepiece.

FIG. 2. APPARATUS FOR DETERMINING HYDROGEN-ION CONCENTRATION COLORIMETRICALLY

One side of the eyepiece has been removed to show the prisms in place. Two tube holders, fastened together as they would be in using the double-tube standards, are shown just above the eyepiece. The blocks with square holes through them are for shutting out side light when only a single-tube holder is used before each opening of the eyepiece. A still larger opening is cut from the upright of the apparatus in front of the cyepiece and covered with a ground glass which diffuses the light and makes it much more satisfactory for color observations.
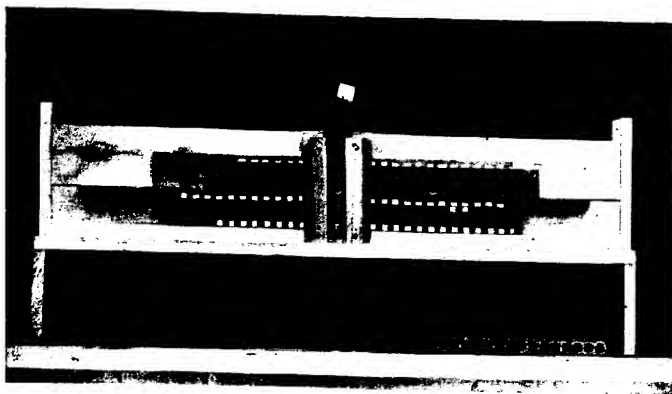
FIG. 1
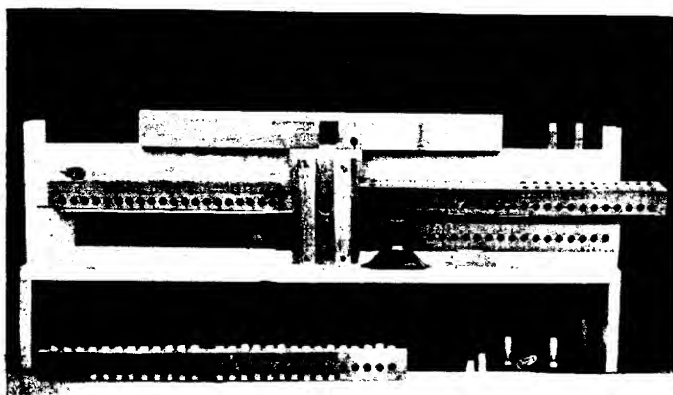
FIG. 2

THE HYDROGEN-ION CONCENTRATION OF CERTAIN THREE-SALT NUTRIENT SOLUTIONS FOR PLANTS

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Until quite recently, practically all of the nutrient solutions used in the study of plant-nutrition problems have contained four or more salts in addition to a trace of iron. In 1915, Shive (4, 5) found that it was possible to make a satisfactory nutrient solution in which all of the essential ions (except iron) were derived from the three salts, calcium nitrate, magnesium sulfate, and monopotassium phosphate.

This at once suggested the possibility that the six essential ions might be derived from other salts and in 1918 Livingston and Tottingham (3) published a paper giving the preliminary results obtained by the use of a three-salt nutrient solution different from that employed by Shive.

These writers suggest six possible ways in which the six essential ions may be put into nutrient solutions by the use of only three salts. The six types of solutions suggested by Livingston and Tottingham are constituted as follows:

TYPE 1	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

In this preliminary paper these writers give a brief account of a study of the variation in yield of wheat seedlings grown in a series of type III solutions, one of Shive's solutions being used as a check.

Since the publication of his original paper, Shive and others have published the results of numerous investigations in which the three-salt solutions were employed both in sand and in solution cultures. As a result of these early investigations the Division of Biology and Agriculture of the National Research Council appointed a special committee to organize a movement for the co-operative study of plant nutrition problems and thus hasten the acquisition of knowledge concerning the salt requirement of a few representative crop plants. In order better to correlate the work of the co-operating scientists this committee formulated standardized methods to be employed and provided each co-operator with a printed copy (2) of directions in which the preparation of the six type-solutions is given in detail.

During the past two years this laboratory has been employing in sand culture studies, solutions of types I, III and IV made up in accordance with the directions prepared by the committee. In studying the plant response to various molecular proportions of the salts included in these solution types it was suggested that it might be possible to correlate the relative growth rates with the hydrogen-ion concentration of the nutrient solutions. Accordingly a series of solutions of each of the six types was made up in accordance with the committee's directions and the hydrogen-ion concentration determined by the method described by Gillespie (1).

In every case, the determination of the hydrogen-ion concentration of the nutrient solutions was made on fresh samples since it was found that the storing in ordinary glass containers for even a day changed the pH values quite materially.

DATA AND DISCUSSION

The pH values of the nutrient solutions are given in table 1. For convenience in discussing the results, they are also arranged in diagrammatic form

TABLE 1

The hydrogen-ion concentration of certain three-salt solutions

SOLUTION NUMBER	HYDROGEN-ION CONCENTRATION EXPRESSED IN TERMS OF pH VALUES					
	Type I	Type II	Type III	Type IV	Type V	Type VI
R_1S_1........................	4.7	3.5	4.1	4.1	3.5	5.3
R_1S_2........................	4.7	3.5	3.7	3.7	3.5	5.2
R_1S_3........................	4.8	3.6	3.6	3.6	3.5	5.2
R_1S_4........................	4.8	3 6	3.6	3.6	3.6	5.2
R_1S_5........................	4.8	3.7	3.6	3.6	3.9	5.2
R_1S_6........................	4.7	3.8	3.5	3.6		
R_2S_1........................	4.6	3.5	4.1	4.1	3.5	5.1
R_2S_2........................	4.6	3.6	3.7	3.7	3.5	5.0
R_2S_3........................	4.6	3.6	3.6	3.6	3.6	5.0
R_2S_4........................	4.6	3.7	3.6	3.6	3.6	5.0
R_2S_5........................	4.6	3.8	3.5	3.5	4.3	
R_3S_1........................	4.5	3.6	4.1	4.1	3.5	4 9
R_3S_2...	4.5	3.6	3.7	3.7	3.6	4.9
R_3S_3	4.5	3.7	3.6	3.6	3.6	4.9
R_3S_4........................	4.5	3.8	3.6	3.6	4.3	4.9
R_4S_1........................	4.4	3.6	4.1	4.1	3.6	4.7
R_4S_2........................	4.4	3.7	3.7	3.7	3.6	4.7
R_4S_3........................	4.4	3.8	3.6	3.6	4.1	4.7
R_5S_1........................	4.4	3.7	4.1	4.1	3.6	4.7
R_5S_2........................	4.4	3.8	3.7	3.7	4.1	4.7
R_6S_1.........'	4.5	3.8	3.9	4.1	3.8	4.8
Control $IR_{3.77}S_{1.93}$ (1.75 atm.)....	4.4					

in figure 1. In fact, it is only when arranged in triangular form that the effect of the volume-molecular proportions upon the H-ion concentration becomes apparent.

Figure 1 brings out the fact that the H-ion concentration is, in general, a function of the volume-molecular proportion of the di-hydrogen phosphate salt used. In other words, as a general rule, all solutions in any one type having the same volume-molecular proportion of the phosphate salt, also have about the same H-ion concentration. It is also to be noted that the types containing KH_2PO_4 are considerably less acid than those containing $Mg(H_2PO_4)_2$ or $Ca(H_2PO_4)_2$. The sulfate and nitrate salts apparently play only a minor part in determining the reaction of the nutrient solutions.

It is not intended to give in this paper a detailed discussion of the influence of the H-ion concentration of nutrient solutions on plant growth. Judging from the results secured at this station with types I, III and IV, the variations in plant growth within any one type cannot be attributed to the hydrogen-ion concentrations of the nutrient solutions. Such parallelism between growth and reaction as exist, seem to be entirely incidental. This does not mean, however, that the H-ion concentration is not a factor in plant growth.

SUMMARY

1. In general, within any one type, the hydrogen-ion concentration of the solution is a function of the volume-molecular proportion of the di-hydrogen phosphate salt present.

2. The types containing KH_2PO_4 have a lower hydrogen-ion concentration than those containing either $Mg(H_2PO_4)_2$ or $Ca(H_2PO_4)_2$.

3. The sulfates and the nitrates apparently play only a minor part in determining the reaction of the nutrient solution.

4. The variations in plant growth, within any one type of solution can not be correlated with differences in the hydrogen-ion concentrations of the solutions.

REFERENCES

(1) GILLESPIE, LOUIS J. 1920 Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. *In* Soil Sci., v. 9, no. 2, p. 115–136.

(2) LIVINGSTON, B. E. 1919 A plan for co-operative research on the salt requirements of representative agricultural plants. Edited by B. E. Livingston, Laboratory of Plant Physiology, Johns Hopkins University, Baltimore, Maryland.

(3) LIVINGSTON, B. E., AND TOTTINGHAM, W. E. 1918 A new three-salt nutrient solution for plant cultures. *In* Amer. Jour. Bot., v. 5, no. 7, p. 337–346.

(4) SHIVE, J. W. 1915 A study of physiological balance in nutrient media. *In* Physiol. Res., v. 1, p. 327–397.

(5) SHIVE, J. W. 1915 A three-salt nutrient solution for plants. *In* Amer. Jour. Bot., v. 2, p. 157–160.

Fig. 1. Diagrams Representing the Hydrogen-Ion Concentration of Six Types of Three-Salt Nutrient Solutions

The number within each circle represents the pH value of that particular solution. The bottom line of each triangle is the base line for potassium, the left side the base line for calcium and the right side the base for magnesium. Each of the three base lines represents a row of solutions each of which has one-eighth of its total volume-molecular concentration derived from the salt for whch it is named, the salt proportion increasing by increments of one-eighth from row to row until the apex of the triangle is reached. The solution at each apex has six-eighths of its total concentration derived from the salt indicated at the opposite base line. For example, the solution represented by the third circle from the left in the second row (type I) has two-eighths of its total concentration derived from mono-potassium phosphate; three-eighths from calcium nitrate and three-eighths from magnesium sulfate.
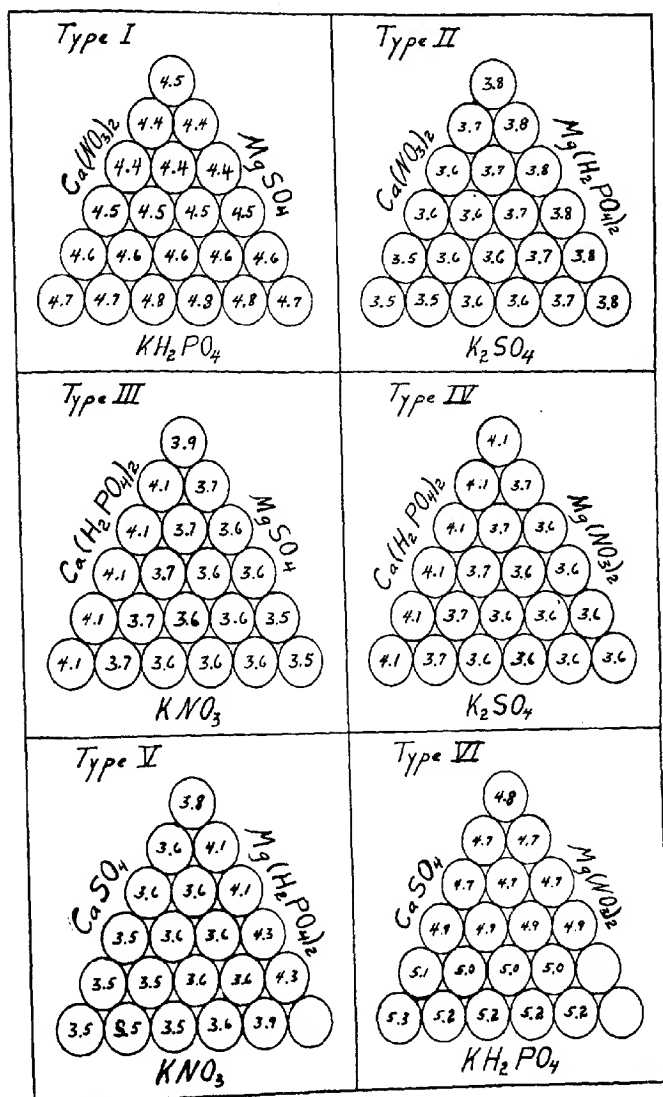
Fig. 1

THE CONCENTRATION OF SODIUM NITRATE TOLERATED BY TOBACCO PLANTS

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It is generally accepted that the nitrate radical is the most favorable source of nitrogen for assimilation by the higher plants and that a proper amount of nitrate is favorable to their growth. On the other hand, too much nitrate is known to be toxic. Nitrates are often used in fertilizers, sodium nitrate (nitrate of soda, or Chile saltpeter) being the one most commonly employed.

In transplanting tobacco to the field by machine, the machine delivers a small quantity of water about the roots of each plant as it is being set in the ground. It has occurred to some growers that the introduction of a little nitrate of soda into the water used in transplanting might serve to give the plant a better start. The literature seems to afford no data upon such a procedure and accordingly the department of agronomy of this station through Mr. E. J. Kinney, this summer began a series of field experiments in which water containing stated amounts of nitrate of soda was applied to tobacco plants as they were set in the field, for the purpose of testing the matter in a practical way, by ascertaining the effect upon the finished crop. The next day after the plants were set a very striking contrast was presented in the appearance of the several lots, those which had received nitrate being more or less wilted, the degree of wilting varying directly with the amount of nitrate received, while those which received none were fresh and thrifty-looking. In course of time, the plants which received the smaller amounts of nitrate recovered, but those which received the largest amount finally died.

It occurred to us that this effect was dependent upon the concentration of sodium nitrate in the solution applied and that some laboratory experiments along this line would be desirable. The results of these have been so positive and striking that we have decided to present them in advance of the agronomy department's report of the field work.

In planning the field experiments, the several quantities of nitrate of soda were adjusted in pounds per acre, as is the custom in applying commercial fertilizers, the amounts being at the rate of 25, 50, 75, 100, 150 and 200 pounds per acre, but the several quantities were dissolved in equal amounts of water, so that the concentration of the solutions increased with the amount per acre. By calculation upon the quantity of water taken, it developed that the concentrations used in the field experiments were equivalent to 25, 50, 75, 100, 150 and 200 parts of nitrate respectively, dissolved in 3750 parts of water

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by weight. Accordingly, the laboratory experiments were planned to cover this range and to include some weaker dilutions.

The experiments were conducted in a good light, near windows, and evaporated water was replaced from time to time with distilled water which had been aerated.

Incidentally it was noted that green algae appeared in some of the flasks, their growth varying roughly with the amount of nitrate added, and that bacteria developed strongly in those containing the higher concentrations, in which the tobacco plants died.

EXPERIMENT A

In this experiment the following dilutions were used:

No. 1—Distilled water.
No. 2—Tap water.
No. 3—1 part commercial sodium nitrate: 3750 parts tap water.
No. 4—5 parts commercial sodium nitrate: 3750 parts tap water.
No. 5—25 parts commercial sodium nitrate: 3750 parts tap water.
No. 6—150 parts commercial sodium nitrate: 3750 parts tap water
No. 7—200 parts commercial sodium nitrate: 3750 parts tap water.

The record of the experiment follows:

June 21, 1920, 2:30 p.m.   Forty young tobacco plants averaging 10 inches from tip of longest leaf to top of roots, were placed on a table to wilt.   Owing to cool and rainy weather the plants had not wilted appreciably at the expiration of 3 hours, the time for closing the laboratory; they were therefore left over night.

June 22, 1920, 8:00 a.m.   All plants were very much wilted.   9:00 a.m.   One of these wilted plants was placed in each of 7 pint jars containing the above solutions, the roots immersed.

June 23, 1920.   Plants no. 1, 2, and 3 had straightened up; leaves firm.   No. 5 less firm and slightly drooping.   No. 4, 6 and 7 drooping and less firm than any.

June 30, 1920.   All plants straightened up and all seem comparable in vigor and firmness except no. 7.   No. 2 and 3 have best new root development.   No. 4 and 1 have small new root development.   No. 5 and 6 have no new root development.   No. 7 wilted and no root development.

July 6, 1920.   All have developed as in the order of June 30.   Practically no root development in no. 5 and 6.   No. 7 dead.

July 13, 1920.   Vigor and development best in no. 3, next in no. 2 and then in no. 1. No. 4, 5 and 6 drooping.

July 20, 1920.   No. 3 decidedly best.   No. 2 and 1 are next best in the order named.   No. 4, 5 and 6, growth arrested.

July 27, 1920.   No. 3 decidedly best.   No. 2 and 1 next best in the order named.   No. 4, 5 and 6 dead; practically no root development.

August 25, 1920.   Relative development of no. 3, 2 and 1 the same as on July 27, 1920.   The vigor and growth of the plants accompany the root development.
Experiment discontinued August 25, 1920.

From this experiment it would seem that no. 3, or a concentration of 1 part of sodium nitrate to 3750 parts of tap water gives the best resulting growth and that higher concentrations show toxic effects.

EXPERIMENT B

The next experiment is recorded below:

June 22, 1920, 8:50 a.m. Planted 7 of the wilted plants from the same lot as those in experiment A, in separate pots containing sand and moistened the sand with solutions of the same concentrations as were used in experiment A.

June 23, 1920, No. 3, 2 and 1 straightening up. No. 4, 5 and 6 drooping; degree about equal. No. 7 very much wilted.

June 30, 1920. Vigor and development best in no. 3; no. 2 next; no. 4 and 1 slightly less. No. 5 and 6, no growth. No. 7 dead.

July 6, 1920. No. 3 decidedly best; no. 2 next, then 1 and 4, in order. No. 5 and 6, no growth.

July 13, 1920. No. 3 best; no. 2 next and then no. 1. No. 4, 5 and 6 have not changed since July 6.

July 20, 1920. No. 3 and 2, development good, about equal; no. 1 much less. No. 5 and 6 dead. No. 4 alive but very little growth since planted.

July 27, 1920. Only small growth in no. 3, 2 and 1 since July 20, 1920; relative increase in no. 3 and 2 is the same. Small growth in no. 1. No further growth in no. 4.

August 25, 1920. No. 3 and 2 have growth and vigor about the same and are 10 inches high. No. 1 about 5½ iches high. No. 4 still alive but no growth.

Experiment discontinued August 25, 1920.

In this experiment plant no. 3, representing a concentration of 1 part of sodium nitrate to 3750 parts tap water, shows the best early growth, indicating marked stimulating effects during that period. Its ultimate growth was better than that of any other receiving the sodium-nitrate solutions and slightly better than that of the plant receiving tap water alone. Higher concentrations were toxic to the plants.

EXPERIMENT C

The results obtained in experiment C are next presented:

June 22, 1920. Two unwilted plants were placed in each of seven 500-cc. flasks containing the same solutions as were used in experiments A and B.

June 23, 1920. No. 1, 2 and 3—conditions unchanged—firm and upright. No. 4 and 5 drooping. No. 6 and 7 drooping badly.

June 30, 1920. Vigor and development of no. 1, 2, 3 and 4 about the same; all erect. No. 5 drooping. No. 6 and 7 dead. Root development, no. 2, 3 and 4 best, no. 1 and 5 less.

July 6, 1920. Relative conditions the same as on June 30, 1920. Only small growth in any since that date.

July 13, 1920. Vigor, growth and root development best and about equal in no. 2 and 3; no. 4 slightly less; no. 1 and 5 still less.

July 20, 1920. Growth and vigor in no. 2 and 3 good and about equal; no. 4, 5 and 1 less in order named.

August 25, 1920. Relative conditions the same. Only small growth in any since July 20, 1920.

Experiment discontinued August 25, 1920.

It will be noted that no. 2 and 3 showed approximately equal development at the end of this experiment. No. 3, grown in the solutions containing 1

part of sodium nitrate to 3750 parts of tap water, showed no initial wilting such as occurred in those receiving the sodium-nitrate solutions of higher concentrations. The higher concentrations were also toxic to the plants receiving them.

<div align="center">EXPERIMENT D</div>

In the experiments described we have shown that the maximum growth was obtained in all cases by no. 3, or the plants receiving a solution of 1 part of sodium nitrate in 3750 parts of tap water, and that solution no. 4 which contained 5 parts of sodium nitrate to 3750 parts of tap water, had the lowest concentration that showed a wilting or toxic effect on the plants. In the case of experiments A, B and C, plants no. 4 showed a wilting effect which was temporary in nature, being completely overcome at a later period; however, their ultimate development did not equal that of the plants which received 1 part of sodium nitrate to 3750 parts of tap water. The latter showed no early wilting effect. To determine the exact strength of solutions which would cause no early drooping or subsequent toxic effect, yet have the early stimulating effect of sodium nitrate, the following experiment was planned.

The concentrations of the solutions were as follows:

No. 1—Tap water.
No. 2—1 part sodium nitrate: 3750 parts tap water.
No. 3—2 parts sodium nitrate: 3750 parts tap water.
No. 4—3 parts sodium nitrate: 3750 parts tap water.
No. 5—4 parts sodium nitrate: 3750 parts tap water.
No. 6—5 parts sodium nitrate: 3750 parts tap water.

The progress of the experiment is here reported:

June 24, 1920. Two unwilted plants about 10 inches from tip to tip were placed in each of 6 flasks containing 400 cc. of the above-named solutions.
June 25, 1920. No. 1, 2 and 3 not drooping and apparently equal in vigor. No. 4, 5 and 6 drooping.
June 28, 1920. All have straightened up and are apparently equal in vigor.
June 30, 1920. No. 3 and 4 best developed; no. 5 next; no. 1 and 2 next and about equal; no. 6 next,
July 6, 1920. Relative size, vigor and development unchanged. Root development corresponding to vigor and development.
July 20, 1920. Vigor and development about the same as on July 6, 1920, except no. 6 drooping. Root development about the same as above; very little root development in no. 6, if any.
August 25, 1920. Relative conditions unchanged since July 20, 1920. Very little growth since that date.
Experiment discontinued August 25, 1920.

From experiment D it will be seen that solutions no. 2, or those receiving 1 part of sodium nitrate to 3750 parts of tap water, caused no temporary wilting while those of stronger concentrations did. It is also indicated from

this experiment that the plants receiving 2 or 3 parts of sodium nitrate per 3750 parts of tap water grew the best, even though they exhibited a temporary wilting at the beginning.

From the foregoing experiments the following conclusions may be drawn:

1. Sodium-nitrate solutions in concentrations greater than 1 part to 3750 parts of tap water cause a wilting which varies in intensity with the concentrations.

2. Concentrations of 150 parts of sodium nitrate to 3750 parts of tap water and higher, cause a wilting which is more or less permanent in character.

3. It is indicated that solutions containing 2 or 3 parts of sodium nitrate in 3750 parts of tap water give the best general development, even though there is a drooping at the outset.

4. When using a solution of sodium nitrate in setting tobacco plants or for plant-beds, its concentration should be considered rather than the amount of nitrate per acre. The latter might be controlled, however, by regulating the amount of solution applied, though with the larger concentration indicated in paragraph 3, allowing a pint to each plant, and 8000 plants to the acre, the amount of nitrate applied would be only 6.4 pounds per acre. The logical method for plant beds would be to make several applications at intervals.

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